中華農業研究

第三十三卷　　第一期

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中華民國七十三年三月

臺灣省農業試驗所出版

中華農業研究

第三十三卷　　第一期

中華民國七十三年三月出版

發行人：萬　雄

總編輯：廖慶樑

編輯委員：李　良　沈再發　邱瑞珍　林益昇　林家棻
林學正　連　深　陳敏祥　黃眞生　蔡新聲
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出版者：臺灣省農業試驗所
臺中縣霧峰鄉萬豐村中正路189號

印刷者：臺灣省政府印刷廠
臺中縣大里鄉中興路一段288號

JOURNAL OF

AGRICULTURAL RESEARCH OF CHINA

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Jour. Agric. Res. China (中華農業研究) 33(1) : 001~011 (1984)

Hybrid Variety of *Indica* Rice and Its Yield Potential[1]

Chen-seng Huang, Ra-hsiung Buu and Chen-chang Chen[2]

Abstract : In a series of testcrosses, none of the varieties was found to restore the CMS of Reimei A (rufipogon), but many restored that of Chen-shan 97A (WA). Hybrid sterility seemed to limit the transfer of Rf-gene from an IR line to a *japonica* variety, Reimei. A row ratio of 4 : 1 or 6 : 1=A : B was found suitable for mechanical planting of A and B or and Rf lines in the hybrid seed production. Chen-shan 97 A, B gave a seedset percentage higher than 30 % or an yield of 1,288 kg/ha for seed production. The amount of hybrid seed produced for the first time in Taiwan was low, 562 kg/ha, which could be raised by increasing the row number of A line and by synchronizing the parental flowering times. Introduced hybrid variety Shan-you 6 gave a high yield of 9.5 t/ha in the first crop, but only about 6 t/ha in the second crop. The yield increase over the control vaiety as performed by the introduced and developed hybrid varieties seemed to be in the range of 20%-30% in the first crop and less than 10% in the second crop.

Introduction

Although cytoplasmic-genetic male sterility in rice was reported as early as 1966 (16) in Japan, followed by the intensive studies made by various researchers in many rice-growing countries (2,3,5,7,8,11,14,17,18,21,22,23), no commercial cultivation of hybrid rice has been known in the free world. Meanwhile, a rice acreage of 4.7 million ha. was reported to be planted to hybrid rice in China mainland in 1978 (15). Various hybrid varieties, including Shan-you 6 (Chen-shan 97A/IR26), inherited the male sterility cytoplasm from *Oryza sativa* L. f. *spontanea* on Hainan island or so-called WA type, and some IR strains were used as the fertility restorers (13). The writer started this line of work in 1974, mostly on *japonica* varieties (4,5,9) and later obtained several varieties of WA type male sterility through IRRI. The results on the development of a hybrid *indica* rice variety in Taiwan and its yield potential are reported here.

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1. Contribution No. 1139 from Taiwan Agricultural Research Institute, supported by the Council of Agricultural Planning and Development.

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Materials and Methods

(1) Testcross for fertility restorers : The first testcross experiment conducted in 1978 resulted in the indentification of 2 Rf-varieties of *indica* rice from about 70 entries tested. They partially restored the fertility of Reimei A (*rufipogon*). The progeny of Reimei A (*rufipogon*)/IR5032 cross was then used to propagate the segregating populations for selecting Rf-lines. In 1980, those lines selected from this IR5032 cross and the new *japonica* strains related to *Oryza rufipogon* were testcrossed (Table 1). Then, various IR strains or lines and other introductions were testcrossed in 1981 and 1982 (Table 1). We used the hot water method to promote the opening of florets and pollinated two panicles per cross with 20-30 florets per panicle. The seedset percentages and grain yields, when fertile, of the F_1's were investigated.

(2) Planting ratio of the CMS A and B lines : Using Chen-shan 97A (WA), B, and other MS varieties which we had developed, planting ratio experiments were conducted in 1981. In the first crop, we tested row-planting ratios of 1 : 1, 1 : 2, 1 : 4=A : B in two replications (Table 2), and then tested the row ratios of 1 : 1, 2 : 1, 3 : 1, 5 : 1=A : B in the second crop (Table 3). No isolation plot was set to avoid pollen contamination in these tests. The seedset percentage and grain yield of the A line were investigated.

(3) Production of hybrid seed : An isolated seed-production plot of hybrid variety rice was set up for the first time in Taiwan in the 1982 first crop. Chen-shan 97A (WA) and B, Toyonishiki A (*rufipogon*) and B, 16-3 A (Boro) and B participated in the MS seed producting. Hybrid rices HRTNGY 1 or Chen-shan 97A (WA)/Suween 293 and HRTNGY 2 or Chen-shan 97A (WA)/IR4563-52-1-3-6 were used for hybrid-seed production. A plot size of about 300 m^2 for Chen-shan 97A, B and its related cross combinations was employed with a row ratio of 5 : 2 for A and B or A and Rf lines. A nylon net at a height of about 2m was set up between different varieties or cross combinations to avoid pollen contamination as much as possible. We adjusted the seedling ages of the A and B lines at transplanting, or adjusted the transplanting dates of A and Rf lines to synchronize their heading stages. Flag leaves were cut back, followed by two sprays of 20 ppm G. A. before heading and then wind-blowing with a power blower during the heading stage to facilitate the dissemination of restorer pollen grains.

(4) Yield test of introduced hybrid varieties : Two hybrid varieties, namely Shan-you 6 and Zha-keng 1 were introduced by the Central Committee Secretariat of the Kuomintang, and the seeds were sent to our institute for tests, via the Council for Agricultural Planning and Development in late July, 1981. We used a large plot size of 13-20m^2 per entry in the 1981 second crop, and 9m^2 in the 1982 first crop for this test. Taichung Sen 3 and Taichung 67 served as the control. Each entry had two plots ; one was single-spaced and the other planted at 3-5 seedlings per hill. Their grain yields, yield components, agronomic characters, and rice quality were investigated. In the 1982 first crop, Shan-you 6 was tested simultaneously in an advanced *indica* rice yield trial.

(5) Yield test of the developed hybrid varieties.

The testcross F_1's that showed a normal seedset were simultaneously used to estimate

their yield potentials for a quick screening, although the number of plants per cross ranged mostly between 20-60. Based on these data, two combinations were selected, i. e. HRTNGY 1 (Chen-shan 97A/Suweon 293) and HRTNGY 2 (Chen-shan 97A/IR4563-52-1-3-6) for seed production in the 1982 first crop season. Then, the two hybrid varieties participated in an observational test. They were single-spaced and also planted at 3-5 seedlings per hill in the 1982 second crop. For a more accurate test of hybrid variety grain yields, we selected 20 cross combinations based on the performances of the F_1's and produced hybrid seeds by hand mass-pollination in the 1982 first crop, then, yield-test was conducted in the following season in a randomized complete block design with four replications. Grain yield and other related characters were investigated according to the method used in the ordinary advanced yield trial.

Results and Discussion

(1) Testcross for fertility restorer :

None of the lines selected from the cross of Reimei A (*rufipogon*)/IR5032 restored the fertility of Reimei A (*rufipogon*), although some lines did to a slight degree (Table 1). Nor did the breeding lines related to *Oryza rufipogon* restore the fertility (Table 1). We backcrossed the F_1 of Reimei A (*rufipogon*)/IR5032 to Reimei using Reimei as the pollen parent. The plants with Rf-gene did not recover in the BC_1F_1. It seems that hybrid sterility limited the transfer of Rf-gene from IR5032 (*indica*) to Reimei (*japonica*). Some of the testcrossed lines related to IR5032, on the other hand, restored completely the fertility of I-geo-gen A (Boro), whose sterility had been relatively unstable, and also restored the fertility of Chen-shan 97A (WA)(Table 1). In the 1981 and 1982 testcrosses, many Rf-varieties were identified as being able to restore the fertility of Chen-shan 97A (WA). Most of them were IR strains that were related to a wild species of *Oryza*. Generally, the CMS's of Chinsurah Boro II and Pai-ko were relatively unstable under Taiwan conditions[5], while those of *rufipogon* and *spontanea* or WA type

Table 1. Seedset percentages of the testcross F_1's for fertility restorers.

Testcross Parents		TC F_1 seedset percentage										
♀	♂	5	15	25	35	45	55	65	75	85	95	Total
Reimei A (*rufipogon*)	*O. rufipogon* related strains	17										17
	IR5032 related lines	22	11	2								35
	others	5										5
I-geo-gen A (Boro)	IR 5032 related lines			2	2	5	3	6	12	8		38
Chen-shan* 97 (WA)	IR5032 related lines	4	8	8	8	8	10	3	11	2	1	63
	1981-II test	11	20	12	12	12	9	22	27	29	7	161
	1982-I test	38	31	15	15	18	11	27	39	33	15	242

*Also spelling Zhen-shan.

Table 2. Seedset percentage of *A* line of the CMS variety at three planting ratios of *B* to *A* lines (1981 1st crop).

Planting ratio	Chen-shan 98A (WA)		Reimei A (rufipogon)		16-3A (Boro)	
A : B	Bagged	Unbagged	Bagged	Unbagged	Bagged	Unbagged
1 : 1	0.49	22.0	0.15	7.0	2.3	8.0
1 : 2	0.51	24.4	0.57	4.7	1.5	7.8
1 : 4	1.80	23.4	0.00	5.3	1.6	7.9

were stable.

(2) **Planting ratio of the CMS A and B lines :**

An increase in the row number of B line did not improve the seedset percentage of the A line in three CMS varieties tested. We can say that an increased supply of pollen grains did not promote the chance of cross-fertilization. Unbagged Chen-shan 97A (WA) showed a higher outcrossing rate of 22%-26%, while two other *japonicas* had a rate of less than 8% (Table 2). Ten to 20 percent of the seeds harvested from these A lines produced plants which exhibited off-type characteristics, indicating that they were the results of pollen contamination. Chen-shan 97 had its stigma protruding outside, even after the floret had closed, and also a logner period of floret opening. One half of the protruding stigma after the floret closed was found to be functional for fertilization for four hours in the morning [9]. Apparently, the flowering habit and flower structure of Chen-shan 97 contribute to this higher rate of outcrossing.

This conclusion can be supported by another CMS variety, Reimei A (*rufipogon*) which has a very low outcrossing rate (Table 2), since its stigmas are very small and never protrude outside the floret or glumes. The period of its floret opening is very short and the frequency of floret opening is also low.

On the other hand, an increase in the row number of A line in a range of 1—5 with one row of B line did not reduce the seed production of CMS varieties (Table 3). Accordingly, yields of hybrid seed increased proportionately as the row number of A line incceased, within the range of row ratios A : B=1 : 1 to =8 : 2 [13]. A row ratio of 6 : 1= A : B is widely used for seed production in China mainland [13]. We used a ratio of 5 : 2 or 4 : 1=A : B in the production of hybrid seed for experimental purposes. For commercial production of hybrid seed, it is recommended to use a ratio of 4 : 1 or 6 : 1=A : B in order to accomodate the rice transplantor.

A high amount of MS seed produced by 16-3A (Boro) and IGGA (Boro) (Table 3) was caused by self-fertilization, since their bagged panicles also showed a higher seedset percentage. As mentioned before, MS lines with Chinsurah Boro II cytoplasm were unstable in male sterility. Therefore, we concentrated thereafter on Chen-shan 97A (WA) for hybrid seed production.

Table 3. Amount of MS-line seed production (g/10m^2) at different planting ratios of *B* to *A* lines (1981 2nd crop).

Planting ratio A : B	Chen-shan 97A (WA)	16-3A (Boro)	34-60A (Boro)	IGG A (Boro)
1 : 1	94	100	29	1,128
2 : 1	135	150	29	857
3 : 1	67	203	20	943
5 : 1	91	115	25	1,355

Table 4. Seedset percentage and seed production of CMS-lines and hybrid variety with a row ratio of A : B=5 : 2 (1982 1st crop).

Item	2.4-D treatment	Chen-shan 97A (WA)(♀)	Chen-shan 97B(♂)	Chen-shan 97A (WA)(♀)	Suweon 293Rf(♂)
Seedset percentage	−	33.6	85.8	18.0	76.0
	+	7.2	75.7	—	—
Seed production(kg/ha)	−	1,288	—	562	—
	+	587	—	—	—

(3) Hybrid seed production

Two steps are needed in hybrid seed production ; one is the MS seed production and the other, hybrid seed. Of three CMS varieties that participated in the seed production, only Chen-shan 97A, B (WA) gave a seedset percentage higher than 30 % and a production rate of 1,288 kg/ha seed (Table 4). Two other *japonica* varieties, Toyonishiki A (*rufipogon*) and 16-3A (Boro), showed a seedset percentage lower than 5 %. Unless some measures are taken to increase their outcrossing rates, the two varieties are practically useless for hybrid variety development. In an attempt to develope such a measure, we sprayed the CMS A lines with 1/800 2,4-D before blooming. The chemical did maintain the opening of florests for more than 3 days. However, these opened florets turned out to be pseudograin-like and greatly reduced the seedset percentage and the amount of seed produced (Table 4).

The amount of hybrid seed produced for the first time in Taiwan was very low, 562 kg/ha in HRTNGY 1 or Chen-shan 97A (WA)/Suweon 293 (Table 4) and even lower than this in HRTNGY 2. Since the MS and Rf varieties all belong to tropical *indica*, they are sensitive to low temperatures when cold fronts invade. Any variety hit by a cold front at the flower initiation stage will spend a considerable time lagging in panicle heading, which may disturb the synchronization of the flowering times of A and Rf lines.

Therefore, the late planted first crop rice is probably more suitable for hybrid seed production in such *indica* rice.

Since a row ratio of 5 : 2=A : B was used in the above mentioned seed production, we could increase the unit-area yields of MS seed and hybrid seed production by increasing the row number of A lines. Synchronization of the A and B line heading times of a MS variety needs further improvement. The heading time of A line generally is late than B line by about three days, and its time of floret opening is later than that of the B line by about 3 hours every day. By solving these timing gaps in the A and B or A

Table 5. Grain yield and lts related traits of hybrid rice Shan-you 6 and the controls.

Variety \ Traits	Year season	Grain yield: Single plant per hill t/ha	Grain yield: Single plant per hill %	Grain yield: 3-5 seedings per hill t/ha	Grain yield: 3-5 seedings per hill %	Grain No./panicle	Seedset (%)	1,000 grains wgt. (g)	Grain straw ratio	Volume weight (g/L)
Shan-you 6	'81Ⅱ	5.4	102	5.9	101	97	80	27.2	1.12	—
	'82Ⅰ	8.5	125	9.5	127	101	82	27.3	1.17	549
Shan-you 6 progeny	'82Ⅰ	5.7	84	6.3	84	100	63	25.9	0.81	543
Taichung Sen 3 (CK₁)	'81Ⅱ	5.3	100	5.8	100	89	77	26.1	0.98	—
	'82Ⅰ	6.8	100	7.5	100	104	68	26.3	0.96	497
Tainung 67 (CK₂)	'81Ⅱ	5.4	100	5.5	—	89	88	26.6	0.96	—
	'82Ⅰ	7.3	100	7.8	—	89	80	25.2	1.13	543

Table 6. Agronomic traits of Shan-you 6 and the controls.

Ⅱ crops : seeded on 31/Ⅶ, 1981 transplanted on 11/Ⅷ 1981
Ⅰ crops : seeded on 2/Ⅱ, 1982, transplanted on 25/Ⅱ, 1982

Variety \ Trait	Year Season	Maturing stage: Pl. height (cm)	Panicle No.: Single pl. per hill	Panicle No.: 3-5 plants per hill	Growth duration (day): Transpl. to heading	Growth duration (day): Transpl. to maturing	Lodging	Pests	Shattering
Shan-you 6	81Ⅱ	91	11	14	57	93	no	none	easy
	82Ⅰ	100	14	22	78	100	no	none	easy
Shan-you 6 progeny	82Ⅰ	93	14	21	80	114	no	none	easy
Taichung Sen 3 (CK₁)	81Ⅱ	88	11	15	68	104	no	none	easy
	82Ⅰ	93	16	24	98	132	no	none	easy
Tainung 67 (CK₂)	81Ⅱ	94	10	13	65	105	no	none	Intermediate
	82Ⅰ	114	16	23	88	122	no	none	Intermediate

and Rf line flowering habits, we may be able to boost the seedset percentage of A lines to 40% or more, which seems to be economical for commercial production of hybrid rice.

(4) **Yield of the introduced hybrid**

Only two varieties were introduced, i. e. Shan-you 6 and Zha-keng 1. Zha-keng 1 belonging to *japonica* was very early maturing and low in grain yield. The agronomical performance of Shan-you 6 is shown in Table 5 and 6. This hybrid variety, commonly grown in China mainland, produced a yield of rough rice 9.5 t/ha in the first crop, and about 6 t/ha in the second crop (Table 5). Its yield increase over Taichung Sen 3 was 27% in the first and 1% in the secound crops (Table 5). An increase in the number of seedings per hill from 1 to 3-5 resulted in a slight yield increase. In the advanced yield trial of the 1982 first crop, Shan-you 6 yielded as high as 8.5 t/ha, the highest yield of all entries tested. Its yield surpassed that of Taichung Sen 10 by 10.1 percent. The progeny of Shen-you 6 produced a lower yield, due to the reduction of hybrid vigor and seedset percentage (Table 5). Shan-you 6 was very susceptible to low temperatures, which was obvious by the severe discolaration of leaves after cold front invasions, particularly in the late second crop season.

Table 7. Rice quality of Shan-you 6 and the controls (Taichung DAIS).

Quality traits / Variety	Milled %	Head rice %	Grain appearence				
			Length	Shape	Trans-parency	White core	White belly
Shan-you 6	67.7	60.2	MS-M	I	4	0	4
Taichung Sen 3 (CK_1)	72.2	61.4	ML	S	2	1	1
Tainug 67 (CK_2)	72.1	67.0	S	B	3	1	2

Quality traits / Variety	Alkali test		Amylose (%)	Protein (%)	Gel consistency
	Spreading	Gelatinization temp.			
Shan-you 6	5.8	H	27.7	7.5	H
Taichung Sen 3 (CK_1)	6	L	19.6	8.6	L
Tainung67 (CK_2)	6	L	18.8	7.6	L

Note : 1. length 5.51mm=S, 5.51–6.1mm=MS, 6.1–6.61mm=M, 6.61–7.06=ML.
2. Shape : B=broad, I=intermediate, S=slender.
3. Transparency, White core, White belly, all have 0-5 classes.

One thing worth of note is that Shan-you 6 had a higher grain straw ratio than the control variety in both crops, primarily due to its heavy grain weight (Table 5). The photosynthetic efficiency of hybrid rice was higher than that of the parents during

Table 8. Examples of yield data of the testcross F_1's (1982-I)

Crosss combination		No. of plants	Grain yield	
♀	♂		kg/ha	%
Chen-shan 97 (WA)	5040	50	8,333	117
Chen-shan 97 (WA)	IR2071-588-4-4	60	8,481	120
Chen-shan 97 (WA)	Bahagia	43	8,409	118
Chen-shan 97 (WA)	LI-109-1-1	33	9,260	130
Chen-shan 97 (WA)	CAS 209	35	8,698	123
Chen-shan 97 (WA)	IR2058-85-3-3	35	8,222	116
Taichung Sen 10 (CK)		100	7,100	100

most of the growth period, particularly in the tillering stage(13). On the other hand, the respiration intensity of the hybrid rice was lower than that of the parents(13). Shan-you 6 seemed to have a larger green leaf area at every growth stage in the first but not in the second crops and also a bigger grain size, indicating that its sizes of sink and source were much larger than the control varieties in the first crop.

The rice quality of Shan-you 6, as determined by the Rice Quality Laboratory, Taichung District Agricultural Experiment Station, is shown in Table 7. Its milling quality, including the percentage of head rice was relatively poor, with high score readings in white belly and transparency. Since its amylose content reached as high as 27.7 % with a hard gel consistency, the market value of this rice will be much lower in Taiwan.

(5) **Yield of the developed hybrid**

The testcross F_1's not only gave us evidence for identifying fertility restorers, but also provided the preliminary yield data for a quick screening. Table 8 shows the yields of high yielding entries of testcross F_1's grown in the 1982 first crop. The range of their yield increases over Taichung Sen 10 was about 20%-30%. Based on the results obtained from the introduced and developed hybrid varieties, the yield increases of hybrid rice over the control variety seems to be about 20%-30% in the first crop season.

The yield increase of hybrid rice over the control variety in the second crop season was much lower. HRTNGY 1 exhibited an yield increase of only 2%-6% and HRTNGY 2, −7% ~0% over Taichung Sen 10 in the second crop season (Table 9). In the replicated yield trial, many entries gave an yield of more than 8 t/ha. Their yield increases over Tainung 67 reached to more than 30%, which would probably drop to less than 10% when compared with Taichung Sen 10.

The high yield potential of semidwarf *indica* rice has been widely acknowledged after the development of IR8, for which Taichung Native 1 serves as the conceptual variety. This group of cultivar generally exhibit a high yield potential under favorable environments. Therefore, the regression coefficients between environment mean and variety

Table 9. Observational trial of 2 hybrid varieties of rice (1982 2nd crop).

Entry	Parents	No. of seedings per hill	Grain yield kg/ha (%)	Plant hgt. (cm)	Panicle No.	Days from transpl. to harvesting
HRTNGY 1	Chen-shan 97A /Suweon 293	1	9,426(106)	106	9.4	96
		3—5	9,100(102)	107	11.0	93
HRTNGY 2	Chen-shan 97A /IR4563-52-1-3-6	1	8,296(93)	105	8.3	92
		3—5	8,900(100)	111	12.0	96
Taichung 10 (CK_1)		3—5	8,889(100)	107	8.9	100
Tainung 67 (CK_2)		3—5	6,667	107	6.7	100

Table 10. Replicated yield trial of hybrid strains of rice (1982 2nd crop).

Entry No.	Parents	Grain yield (kg/ha)	Days from tranpl. to flower.	Plant height (cm)	Panicle No.	Seedset %	No. grain/ panicle	1000 grain wgt. (g)
1	Chen-shan 97A/IR28	7,696	51	97	16	88	94	25.4
2	Chen-shan 97A/IR30	8,689	62	97	14	78	130	24.7
3	Chen-shan 97A/IR50	8,402	52	97	17	81	106	23.5
4	Chen-shan 97A/IR9129 -209-2-2-2-1	8,278	61	100	17	87	116	24.7
5	Chen-shan 97A/IR4563	7,744	63	98	14	82	110	28.0
6	Chen-shan 97A/IR13240	7,942	50	88	16	82	106	25.0
7	Chen-shan 97A/Suweon 293	8,204	64	97	15	71	124	25.5
8	Chen-shan 97A/9719	7,487	57	100	16	86	124	24.3
9	Chen-shan 97A/9733	7,364	59	101	12	76	133	30.2
10	Tainung 67	6,529	65	107	17	86	100	24.2

mean yields are generally higher; or their yield potentials are high, but they have a high regression coefficient or low general stability[10]. In the hybrid variety produced from these semidwarf *indicas*, there are two possibilities concerning environmental adaptation : the first is that the heterozygosity and uniformity of the hybrid variety bring about an improvement in its stability, so that it adapts better to a wider range of different environments; and the second is that the hybrid vigor of yield potential manifests itself even higher under favorable environments, but not under unfavorable environments, therefore, its general stability is low.

From the above results, it seems that the second possibility is more likely. We recommended two hybrid strains, namely HRTNGY 1 and 2 to participate in the *indica* regional yield trial in 1983. More accurate conclusions dealing with the environmental adaptation of hybrid rice will be drawn after the completion of this trial.

Literature Cited

1. Anonymous, 1980. Hybrid rice. In IRRI Annual Report for 1980, pp. 143-146.
2. Athwal, D. S. and S. S. Virmani. 1972. Cytoplasmic male sterility and hybrid breeding in rice. Rice Breeding, IRRI, Los Banos, phillipines, pp. 615-620.
3. Carnahan, H. L., J. R. Erickson, S. T. Tseng and J. N. Rutger. 1972. Outlook for hybrid rice in the U. S. A. Rice Breeding, IRRI, Los Banos, philipines, pp. 603—607.
4. Cheng, Y. K. and C. S. Huang. 1978. Some properties of cytoplasmic male sterile rice. J. Agr. Res. (Taiwan) 27, 267-290 (in Chinese with English summary).
5. Cheng, Y. K. and C. S. Huang. 1979. Studies on cytoplasmic-genetic male sterility in cultivated rice (*Oryza sativa* L.). I. Effect of different cytoplasm sources on male abnormalities at anthesis. J. Agr. Assoc. China (Taiwan) N. S. 106, 1-22.
6. Chu, Y. E., K. S. Tsai and H. W. Li. 1970. Hybrid rice breeding. I. Variation of flowering time and duration. Bot. Bull. Acad. Sinica 11, 105-112.
7. Erickson, J. R. 1969. Cytoplasmic male sterility in rice (*Oryza sativa* L.). Agron. Abstr. p. 6. U. S. A.
8. Hoff, B. J. and D. Chandrapnya. 1973. Inheritance of two male sterile characters in rice. Agron. Abstr. 65th Ann. Meet. American Soc. Agron. p. 7.
9. Huang, C. S. 1977. An appraisal of rice breeding technique. J. Agr. Assoc. China (Taiwan). N. S. 100, 5-21.
10. Huang, C. S. and H. P. Wu. 1979. Environmental adaptation of rice varieties. Proc. ROC-Japan Symp. on Rice Productivity. Monograph Series No. 3, pp. 29-38, Inst. Bot, Academia Sinica, Taipei .
11. Kitamura, E. 1962a. Studies on cytoplasmic sterility of hybrids in distantly related varieties of rice, *Oryza sativa* L. II. Fertility of the F_1 hybrid between strains derived from certain Philippine×Japanese variety crosses and Japanese varieties. Jap. J. Breed. 12, 81-84.
12. Kitamura, E. 1962b. Studies on cytoplasmic sterility of hybrids in distantly related varieties of rice. *Oryza sativa* L. II. Analysis of nuclear genes in Japanese varieties controlling cytoplasmic sterillity. Jap. J. Breed. 12, 166-168.
13. Lin Shan-Cheng and Yuan Long-ping. 1980. hybrid rice breeding in China. In Innovative Approaches to Rice Breeding, IRRI, Los Banos, Philippines, pp. 35-54.
14. Rutger J. N. and C. Shinjyo. 1980. Male sterility in rice and its potential use in breeding. In Inovative Approbches to Rice Breeding, IRRI, Los Banos, Philippines, pp. 53-66.
15. Shan Jin-hua. 1980. Rice breeding in China. In Rice Improvement in China and Other Asian Countries, IRRI, Los Banos, Philippines, pp. 9-30.
16. Shinjyo, C. and T. Omura. 1966. Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. I. Fertilities of F_1, F_2 and offsprings obtained from their mutual reciprocal back crosses and segregation of completely male sterile plants. Jap. J. Breed. 16 (Suppl. 1), 179-180.
17. Shinjyo, C. 1969. Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. II. The inheritance of male sterility. Jap. J. Genet. 44, 149-156.
18. Shinjyo, C. 1972. Distributions of male sterility-inducing cytoplasms and fertility-restoring genes in rice. I. Commercial lowland rice cultivated in Japan. Jap. J. Genet. 47, 237-243.
19. Shinjyo, C, 1975. Outcrossing rate on cytoplasmic male sterile plants of rice in the first and second crop seasons in Okinawa. Jap. J. Breed. 25 (Suppl. 1), 143-144.
20. Shinjyo, C. , R. Nishime and Y. Watanabe, 1974. Inheritance of fertility restoring gene Rfx and Rf

in male sterile cytoplasm derived from variety Lead Rice (Preliminary rept.). Jap. J. Breed. 24 (Suppl. 1), 130-131.

21. Shinjyo, C. and Y. Watanabe. 1977. Allelism test for two genes showing fertility restoring effect for male sterile cytoplasm of Lead Rice and their inheritance. Jap. J. Breed. 27 (Suppl. 2), 70-71.

22. Watanabe, Y.. S. Sakaguchi and M. Kudo. 1968. On the male-sterile rice plant possessing the cytoplasm of Burmese variety, "Lead Rice". Jap. J. Breed. 18 (Suppl. 2), 77-78.

23. Yabuno, T. 1977. Genetic studies on the interspecific cytoplasm substitution lines of Japonica varieties of *Oryza sativa* L. and *O. glaberrima* Steud. Euphytica 26, 451-463.

秈稻雜種品種及其產量潛力[1]

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摘 要

本文報告秈稻雜種品種之育成及其產量潛力。多次檢驗交配結果顯示，現有育成系統、品系或品種均未能恢復 Reimei A (*rufipogon*) 之雄不稔性，但有多數品種（系）恢復 Chen-shan 97A (WA) 之不稔性。秈稉型間雜種不稔性似限制稔性恢復基因從一個 IR 品系轉移至稉型的 Reimei。生產雜種種子所需 A、B 系統或 A、Rf 系統之種植行數比，以4：1或6：1=A：B或 A：Rf較適合於機械插秧。雄不稔品種 Chen-shan 97A, B 有 30% 以上之結實率，種子生產量達1,288公斤／公頃。目前的雜種品種種子生產量低，僅有 562 公斤／公頃，有可能以增加 A 系統之行數及同步化兩親的開花期來將之大幅提高。引進雜種品種汕優 6 號在第一期作產量達 9.5 公噸／公頃，第二期作 6 公噸／公頃。引進及育成雜種品種的一般產量潛力似比對照品種高約20%～30%（第一期作），及10%以下（第二期作）。

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Jour. Agric. Res. China (中華農業研究) 33(1) : 012~023 (1984)

Physiological Studies of Rice Tillers
II. Productivity of Varieties Differing in Grain Volume[1]

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Abstract : Amount of dry matter, total nitrogen and total nonstructural carbohydrates (TNC) in the leaf blades, leaf sheaths and stems from various node positions, as well as panicles, of rice tillers were analyzed at stages of grain development. The purpose of this study was to compare the potential of productivity between a large-grain variety Mira and a medium-grain variety Tainung No. 67. Nitrogen concentrations in various organs of Mira tillers were significantly higher, whereas TNC concentrations were lower, than those of Tainung No. 67 tillers. When compared to Mira on a per tiller basis, Tainung No. 67 produced 14-55% less dry matter and 36-39% less TNC during the test period. For a mature panicle from a single tiller, the amounts of dry matter and TNC accumulated by Tainung No. 67 were only 61-74% and 63-80%, respectively, of those accumulated by Mira. Those results indicated that the productivity per tiller was greater for the large-grain variety. As panicle number per hill was very low for Mira, community productivity was 50% higher for Tainung No. 67 in the first crop but was comparable between varieties in the second crop. No difference in the increase rates of major components in panicles was found between varieties, indicating the absolute amount of translocation into panicles was greater for Mira. Results suggested that yield increase through enlarging grain size was possible. However, responses of the large-grain rice to the environmental factors should be further understood.

In a previous report [13] we found that for the first crop rice grown in Taichung area, up to 20% of total nonstructural carbohydrates in rice tillers remained in the vegetative organs at maturity. We therefore hypothesized that the limitation to the yield of the first crop rice was sink size rather than source capacity, and yield increase of 20% was possible providing that panicle volume could be enlarged to accept the more than enough photosynthates produced by the leaf blades during grain-filling.

Rice yield can be enhanced by increasing the value of any one of the four yield components without simultaneously affecting the performance of the others. Panicle number per hill, which was the main factor in causing the low yield of the second crop rice [14], has been considered as adequate for rice grown in the first crop season. Further improvement might be difficult especially for the machine-transplanted rice with a na-

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1. Contribution No. 1140 from Taiwan Agricultural Research Institute. This study was supported in part by a grant from Council for Agricultural Planning and Development, Executive Yuan, Republic of China.

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rrow spacing of 30×15cm.

Spikelet number per panicle and the percentage of seed-setting are usually negatively correlated[6]. According to Ishizuka[3], 80 spikelets per panicle and seed-setting rate of 85%, along with 400 panicles/m^2 and 1,000-grain-weight of 22g, were ideal for rice yield of 6 t/ha. In Taiwan, it is not uncommon that rice panicle has 100-150 skikelets and over 90% of them are able to bear well-developed seeds in the first crop season. In other words, yield increase through increasing spikelet number per panicle and seed-setting percentage would not be very effective.

The size of rice grains is limited by the surrounding hulls[6]. Thousand-grain-weight of most commercial varieties is around 23-25g. However. seed weight of many large-grain varieties is as high as 40-45mg. Theoretically, an increase of seed weight from 25mg to 30mg means 20% increase in grain yield. The problems remain as whether or not the photosynthetic productivity during the period of grain-filling can meet the demands by the large-volume panicles.

The objective of this study was to examine the potential of dry matter production of a large-grain rice variety on a per tiller and community basis. The distribution of total nonstructural carbohydrates and nitogen among different tiller fractions was studied. A medium-grain commercial variety was also included for comparison.

Materials and Methods

A japonica, large-grain (1, 000-grain-weight about 37g) rice cultivar introduced from the Philippines, Mira, and a japonica, medium-grain cultivar, Tainung No. 67 were grown at the experimental farm of Taiwan Agricultural Research Institute in 1981. In the first crop, transplanting date was Feb. 20 and heading date was April 28 for Mira and May 11 for Tainung No. 67. In the second crop, transplanting was on Aug. 10 and heading on Sept. 25 and Oct. 13 for Mira and Tainung No. 67 respectively. Maturity date was 5 weeks after heading in the first crop and harvest was made 4 weeks after heading in the second crop. The early harvest of the second crop rice wsa due to the heavy infestation of brown plant hopper.

Seedlings were raised in the field until at the leaf age of 4.5-5.0 and transplanted by hand at a spacing of 25×25cm. Plots, sized at 7.5×5m, were arranged in a randomized complete block design with three replications. Before transplanting, N, P_2O_5 and K_2O were applied to all plots at, respectively, 60, 60 and 50kg/ha in the first and 65, 60 and 50kg/ha in the second crop. Additional nitrogen at a rate of 30kg/ha each was topdressed twice in both crops.

At least 8 hills of rice plants including roots were harvest from each plot at a week's interval between heading and maturity. The collected plants were cleaned with tap water and 80 large tillers of each variety were selected and combined. Only the above-ground portion was kept and the samples were further divided according to the position of the nodes on a basipetal basis. The uppermost node was designated as the first node. Leaf blade, leaf sheath and stem from ther same node were separated as individual samples. Tose samples from the 4th and lower nodes were combined. Panicle was divided separately.

Samples were dried in a forced draft oven at 100°C for 2 hrs follwed by 70°C for 24 hrs. After dry weight had been determined, the samples were ground with a Wiley mill through a 40-mesh screen. Concentration of total nonstructural carbohydrates was then determined according to Smith[11]. Nitrogen was determined by the Kjeldahl procedures.

Results on the dry weight of the same plant fraction (leaf blade, leaf sheath or stem) from various node positions were pooled and reported. Comparison between the developmental stages of heading and maturity was emphasized. Changes in concentrations of N and total nonstructural carbohydrates of different plant parts during grain-filling were also discussed in terms of physiological importance.

Results

I. N concentrations in tiller organs during grain-filling

N concentrations of the leaf blades, leaf sheaths. stems and panicles at stages of grain development were indicated in Table 1. It was generally true that the upper leaf blades had higher N concentrations than the lower ones. Leaf blade N percentages were highest at heading and decreased continuously thereafter for either variety in both crops. For rice tillers grown in the first crop season, Mira contained more N in the leaf blades than Tainung No. 67 during the first two weeks of grain development. However, the concentrations were higher in Tainung No. 67 leaves during the subsequent period of grain-filling. In contrary, leaf N was consistently higher for Mira than for Tainung No. 67 up to three weeks after heading in the second crop.

Table 1 also showed that at the stage of heading, leaf N was higher in the first (2.24—3.14%) than in the second (2.20-2.16%) crop for Tainung No. 67 and was comparable between the two crops (2.88-3.52%) for Mira tillers. N concenrtations decreased faster thereafter in the first crop season as they were higher in the second crop between one and four weeks after heading. Final concentrations of N in the leaf blades at four weeks after heading were, respectively, 0.51-1.62% and 1.10 1.99% in the first and second crops for Tainung No. 67. Corresponding values for Mira were 0.85-0.93% and 0.94-1.89%.

Similar pattern of changing N concentrations in the leaf sheaths and stems was recorded (Table 1). N concentrations were higher in the leaf sheaths than in the stems and decreased continuously in both organs towards maturity. The second crop and Mira variety had higher N concentrationts than the first crop and Tainung No. 67, respectively.

Panicle N concentrations showed little fluctuation during grain-filling. The only exception was Tainung No. 67 in the second crop which decreased from 1.22% at heading to 0.88% at harvest. The concentrations at matruity were around 1.3% for Mira which was significantly higher than the 0.88-0.99 % of Tainung No. 67 tillers.

Table 1. Nitrogen concentrations (%, dry basis) in different plant fractions of Tainung No. 67 and Mira rice tillers at stages (weeks after heading) of grain-filling.

Variety	Tiller Fraction[a]	First Crop						Second Crop				
		0	1	2	3	4	5	0	1	2	3	4
Tainung No. 67	1st leaf	3.14	2.70	2.27	1.91	1.62	1.30	2.61	2.89	2.77	2.30	1.99
	2nd leaf	2.69	2.21	1.93	1.51	0.82	0.55	2.50	2.33	2.30	2.06	1.58
	3rd leaf	2.24	1.90	1.56	1.09	0.51	0.50	2.20	2.06	1.95	1.77	1.10
	Leaf sheath	0.64	0.57	0.51	0.43	0.35	0.39	0.69	0.67	0.59	0.49	0.51
	Stem	0.55	0.40	0.32	0.28	0.26	0.31	0.58	0.47	0.37	0.35	0.31
	Panicle	1.04	0.96	0.98	0.96	0.97	0.99	1.22	1.08	1.00	0.93	0.88
Mira	1st leaf	3.36	3.31	2.44	1.22	0.93	0.93	3.52	3.45	3.23	2.72	1.89
	2nd leaf	3.41	2.80	2.34	1.22	0.92	0.87	3.36	3.34	2.83	2.33	1.60
	3rd leaf	2.88	2.41	1.97	0.91	0.85	0.77	2.88	2.73	2.43	1.82	0.94
	Leaf sheath	1.02	0.77	0.71	0.53	0.50	0.51	1.29	1.05	0.85	0.73	0.62
	Stem	0.93	0.58	0.38	0.36	0.41	0.39	1.36	1.25	0.67	0.58	0.51
	Panicle	1.46	1.36	1.35	1.29	1.35	1.38	1.28	1.25	1.35	1.38	1.37

[a]Leaf blades were sequenced basipetally according to the position of nodes on tillers ; combined data of leaf sheaths and stems from various node positions were presented.

II. TNC concentrations in tiller organs during grain-filling

Table 2 showed that concentrations of total nonstructural carbohydrates (TNC) in the leaf blades decreased slowly but continuously during the whole period of grain development. Mira had higher TNC percentages (5.5% and 8.3% in the first and second crops, respectively) than Tainung No. 67 (3.9% and 4.1%) at heading. However, final concentrations at harvest were similar between the two varieties in the first crop (about 2%). It was much higher foe Tainung No. 67 (4.2%) than for Mira (2%) in the second crop.

TNC concentrations were lower in the uppermost and lowest leaf sheaths than in the middle (2nd and 3rd from top) leaf sheaths at any stage of grain-filling. The decreases in TNC concentrations were most pronounced between the first and second weeks after heading in both crops. It was also found that the first crop contained more TNC in the leaf sheaths than the second crop. Tainung No. 67 tillers were also higher in sheath TNC than Mira, especially in the second crop season.

Stems had very high TNC concentrations at heading (Table 2). Highest values were recorded in the lower stem sections, e. g., 40% for Tainung No. 67 and 30% for Mira in the first crop. Those values in the second crop were around 30% and from 13% to 27% for the two varieties, respectively. Marked decreases in stem TNC were also found between the first and second weeks after heading which coincided with the period of rapid grain-filling.

Table 2. Concentrations of total nonstructural carbohydrates (%, dry basis) in different plant fractions of Tainung No. 67 and Mira rice tillers at stages (weeks after heading) of grain-filling.

Variety	Tiller Fraction[a]	First Crop						Second Crop				
		0	1	2	3	4	5	0	1	2	3	4
Tainung No. 67	Leaf blade	3.9	3.4	2.9	1.9	2.3	1.6	4.1	3.8	3.0	2.0	4.2
	1st sheath	6.8	5.3	3.1	1.0	2.0	2.1	6.5	3.5	3.2	1.2	3.1
	2nd sheath	16.1	11.3	6.6	1.6	2.3	1.8	11.5	10.5	6.0	2.5	2.7
	3rd sheath	20.0	13.6	5.4	1.4	1.6	1.0	13.5	13.0	6.5	1.5	1.9
	4th sheath	15.0	9.0	2.8	0.7	1.4	0.6	6.3	6.4	2.8	1.5	1.2
	1st stem	17.1	18.3	7.0	2.3	3.0	2.5	12.3	10.0	10.0	2.8	3.2
	2nd stem	34.5	32.2	25.1	5.8	5.5	9.5	25.2	25.9	25.2	8.2	6.4
	3rd stem	40.3	32.0	25.3	3.8	4.7	11.1	30.2	31.9	25.0	6.6	4.6
	4th stem	40.6	29.1	23.3	2.7	3.5	10.6	31.8	32.3	22.4	4.8	3.0
	Panicle	5.0	26.4	50.5	58.4	61.5	61.6	4.5	9.6	40.6	51.4	58.1
Mira	Leaf blade	5.5	6.4	3.2	3.0	2.6	2.1	8.3	7.1	4.1	3.7	2.0
	1st sheath	6.8	5.4	4.1	4.2	5.3	4.8	5.4	5.0	2.6	2.0	1.6
	2nd sheath	10.8	10.3	6.5	3.3	2.7	2.1	6.8	8.2	2.5	1.8	1.4
	3rd sheath	14.2	15.0	10.1	2.5	3.2	2.6	6.3	6.3	2.6	1.5	1.0
	4th sheath	14.4	12.4	8.2	1.6	1.0	1.0	3.0	3.2	1.5	1.3	0.8
	1st stem	12.2	27.3	9.4	3.2	3.0	2.5	13.0	23.8	5.0	2.1	1.6
	2nd stem	22.5	37.8	19.4	5.5	6.2	5.7	27.6	35.8	7.1	2.4	2.1
	3rd stem	29.8	36.8	24.8	5.3	7.3	7.1	26.7	35.7	7.5	2.7	1.6
	4th stem	31.0	34.4	24.9	6.5	12.7	12.8	13.4	25.1	5.9	2.4	1.2
	Panicle	4.7	22.1	46.2	55.7	55.9	56.0	5.9	10.4	42.7	57.4	58.3

[a]Leaf sheaths and stems were sequenced basipetally according to the position of nodes on tillers; combined data of leaf blades from various node positions were presented.

Panicles just extruded from the embedding leaf sheaths possessed only 5% of their dry matter as TNC. In one week, the concentrations increased to 22-26% in the first but only 10% in the second crop. TNC deposition into panicles of Tainung No. 67 tillers was completed within a duration of 4 weeks in both crops (Table 2). The mature panicle contained 61% of TNC in the first crop and 56% in the second crop. The duration of grain filling for the large-grain Mira tillers was shorter (3 weeks) than that of Tainung No. 67. Final concentrations were 56% and 58% for the first and second crop, respectively.

III. Changes in the amounts of dry matter, N and TNC during-filling

Dry weights of different plant fractions of rice tillers at heading and maturity were shown in Table 3. For the variety Tainung No. 67, total dry weight per tiller at heading was 3,140mg in the first crop and 2,990mg in the second crop. Dry weight increased to 4,480mg at maturity, or a net dry matter accumulation of 1,340mg, for the first crop. The increase was 1,550mg, or 210mg higher, for tillers grown in the second crop season. For Mira, dry weight increased from 3,290mg at heading to 6,250mg at maturity in the first crop and from 4,100mg to 5,900mg in the second crop. Those results indicated that net dry matter production of Mira tillers during grain-filling was higher in the first (2,960mg) than in the second crop (1,800mg).

Table 3. Changes in dry weight (mg/tiller) of Tainung No. 67 and Mira rice tillers from heading to maturity

Variety	Tiller Fraction	First Crop			Second Crop		
		Heading	Maturity	Difference	Heading	Maturity	Difference
Tainung No. 67	Leaf blade	670	550	− 120	820	820	0
	Leaf sheath	1,040	800	− 240	980	990	+ 10
	Stem	870	620	− 250	750	690	− 60
	Panicle	540	2,510	+ 1,970	440	2,040	+ 1,600
	Small tiller[a]	20	0	− 20	0	0	0
	Total	3,140	4,480	+ 1,340	2,990	4,540	+ 1,550
Mira	Leaf blade	830	610	− 220	1,050	770	− 280
	Leaf sheath	1,100	870	− 230	1,100	900	− 200
	Stem	870	1,020	+ 150	1,060	900	− 160
	Panicle	490	3,410	+ 2,920	670	3,330	+ 2,660
	Small tiller[a]	0	340	+ 340	220	0	− 220
	Total	3,290	6,250	+ 2,960	4,100	5,900	+ 1,800

[a]Small tillers originated from above-ground nodes of the sampled large tillers.

Despite the similar total tiller dry weight at heading of the two varieties in the first crop. Mira accumulated 40% more dry matter than Tainung No. 67 at the time of maturity. Panicle weight was also 36% higher for Mira (3,410mg vs. 2,510mg). Average panicle weight of Tainung No. 67 tillers in the second crop was 2,040mg or 470mg lower thna that in the first crop. However, the difference was only 80mg per panicle for Mira tillers between the two rice crops.

The amounts of N present in different plant fractions were shown in Table 4. For either crop or variety, the largest amount of N was recovered in the leaf blades at heading and in the panicles for the mature rice tillers. There was a consistent loss of N from leaf blades, sheaths and stems and a significant N deposition into panicles during the test period. The loss of N from vegetative organs was 11.8-19.0mg per tiller

for Tainung No. 67 and 29.4-37.7mg for Mira. On the other hand, grain of N by developing panicles was 12.6-18.8mg for Tainung No. 67 and 37.0-38.8mg for the large-grain Mira. Comparison between heading and maturity showed that rice tillers contained similar amount of N with the exception of Mira in the first crop where an increase of 12.8mg per tillers was recorded.

Table 4. Changes in the amount of nitrogen (mg/tiller) in Tainung No. 67 and Mira rice tillers from heading to maturity.

Variety	Tiller Fraction	First Crop			Second Crop		
		Heading	Maturity	Difference	Heading	Maturity	Difference
Tainung No. 67	Leaf blade	16.4	4.8	− 11.6	18.3	10.6	− 7.7
	Leaf sheath	6.6	2.8	− 3.8	6.8	5.0	− 1.8
	Stem	4.8	1.6	− 3.2	4.4	2.1	− 2.3
	Panicle	5.6	24.4	+ 18.8	5.4	18.0	+ 12.6
	Small tiller[a]	0.4	0.0	− 0.4	0.0	0.0	0.0
	Total	33.8	33.6	− 0.2	34.9	35.7	+ 0.8
Mira	Leaf blade	23.6	5.1	− 18.5	31.0	10.8	− 20.2
	Leaf sheath	11.3	4.4	− 6.9	12.5	6.1	− 6.4
	Stem	8.1	4.1	− 4.0	10.1	3.6	− 6.5
	Panicle	7.1	46.0	+ 38.9	8.6	45.6	+ 37.0
	Small tiller[a]	0.0	3.3	+ 3.3	4.6	0.0	− 4.6
	Total	50.1	62.9	+ 12.8	66.8	66.1	− 0.7

[a]Small tillers originated from above-ground nodes of the sample large tillers.

The production and distribution of TNC in rice tillers were shown in Table 5. It was obvious that Tainung No. 67 contained more TNC in the mature panicles in the first crop (1,528mg per panicle) than in the second crop (1,184mg). Panicles of Mira, on the other hand, possessed 25% more TNC than those of Tainung No. 67 in the first crop and 59% more in the second crop. Net accumulation of TNC in the whole tiller was also significantly higher for Mira rice, especially in the second crop season. Results also showed that at the time of harvest, 92-98% of TNC present in the tillers had been deposited into panicles, suggesting hign efficieny of utilizing TNC for economic production of the rice varieties tested. Contribution of pre-heading storage to panicle TNC was, respectively. 20% and 15% for Tainung No. 67, and 11% and 13% for Mira in the first and second crops under the assumption that the translocation efficiency was 70%.

Table 5. Changes in the amount of total nonstructural carbohydrates (mg/tiller) in Tainung No. 67 and Mira rice tillers from heading to maturity

Variety	Tiller Fraction	First Crop			Second Crop		
		Heading	Maturity	Difference	Heading	Maturity	Difference
Tainung No. 67	Leaf blade	26	13	− 13	34	34	0
	Leaf sheath	148	15	− 133	85	20	− 65
	Stem	310	26	− 284	211	29	− 182
	Panicle	28	1,528	+ 1,500	20	1,184	+ 1,164
	Small tiller[a]	3	0	− 3	0	0	0
	Total	515	1,582	+ 1,067	350	1,267	+ 917
Mira	Leaf blade	45	16	− 29	88	16	− 72
	Leaf sheath	131	29	− 102	58	11	− 47
	Stem	246	85	− 161	221	15	− 206
	Panicle	23	1,906	+ 1,883	40	1,878	+ 1,838
	Small tiller[a]	0	71	+ 71	15	0	− 15
	Total	445	2,107	+ 1,662	422	1,920	+ 1,498

[a]Small tillers originated from above-ground nodes of the sample large tillers.

IV. Comparison on productivity between varieties on single tiller and community bases

Numbers of panicle per hill at harvest were 16.1 and 14.9 for Tainung No. 67 in the first and second crops, respectively. Corresponding values for Mira were 7.6 and 9.8 (data not shown). Multiplication of the results in Tables 3-5 (based on single tiller study) by panicle number per hill was presumed to be the performance of whole plant. Taking the figures of Mira as 1.00, the ratios of some characters between the medium-grain Tainung No. 67 and large-grain Mira were shown in Table 6.

Table 6. Comparisons on the ratios of some characters between single-tiller and community performances of Tainung No. 67 and Mira rice at maturity[a]

Crop Season	Tiller Fraction	Dry Matter Accumulated		Amount of N Absorbed		Amount of TNC Produced	
		Single tiller	Community	Single tiller	Community	Single tiller	Community
First	Panicle	0.74	1.55	0.53	1.13	0.80	1.70
	Total	0.72	1.51	0.53	1.13	0.75	1.58
Second	Panicle	0.61	0.94	0.40	0.60	0.63	0.97
	Total	0.77	1.16	0.54	0.82	0.66	1.00

[a]Amount of dry matter (N, TNC) in Mira divided by amount of dry matter (N, TNC) in Tainung No. 67.

One Tainung No. 67 tiller yielded 72-77% of dry matter and 66-75% of TNC when compared with Mira tiller. The amount of N present in a Tainung No. 67 tiller at harvest was only 53-54% of that in a Mira tiller. The community performances were reversed. For example, production of dry matter and TNC per hill of plant was 50% more for Tainung No. 67 in the first crop and about the same as Mira in the second crop. Those results indicated higher potential of productivity for single Mira tiller but less production in the actual field circumstance.

Discussion

Generally, N concentrations in the leaf blades were higher in the second than in the first crop during the whole period of grain-filling (Table 1). This was in accordance with the results of Chen *et al* [1] and Wei *et al* [13]. Oritani *et al* [10] reported that photosynthetic rate of rice leaves was positively correlated with concentrations of total N, soluble protein and fraction-I protein. For rice grown in Taiwan, Chen *et al.* [1] also suggested that the composition of N-containing compounds was more important than N concentration to the physiological activity of rice plant.

Although Yoshida [15] estimated that the critical N concentration for a high leaf photosynthetic rate was 2%, the fact that Mira contained more N in the leaf blades and culms than Tainung No. 67 indicated varietal difference in the ability of obtaining N from soils. Table 3 showed that the potential of dry matter production during grain-filling was significantly higher for Mira tillers. Whether or not the two phenomena were interrelated has yet to be determined. However, Tanaka [12] reported that photosynthetic rate of rice leaves was curvilinearly correlated to N concentration up to 3% or higher.

In contrast to the results on N, concentrations of TNC in the leaf sheaths and stems were significantly higher for Tainung No. 67 at the stage of heading, especially in the first crop. Many reports [7,15] indicated an inverse correlation between N and TNC accumulation. However, in view of the marked increase in TNC concentration in Mira stems during the first week of grain development, the relationship between N and TNC was more complex and subjected to the influences of rice growth and differentiation *per se*.

The rapid decline of TNC concentrations in the vegetative organs between 2 and 4 weeks after heading was in good agreement with the rate of TNC accumulation in the panicles. In other words, a relatively large amount of TNC was retranslocated from culms to the developing panicles during this period, as reported by Cock and Yoshida [2]. In the first crop season, TNC concentration of the lower stem sections increased gradually starting from the third week after heading. The continued deposition of TNC into stems indicated clearly that stems had replaced panicles as sink demanding photosynthates from the still active leaf blades. Similar result has been obtained by this laboratory (unpublished data) which has led to the conclusion that the limitation to grain yield was different between the first and second rice crops.

According to Murata and Matsushima [9], all vegetative parts stopped growing after flowering. For Mira rice grown in the first crop of this study, it was found that depo-

sition of dry matter into stems continued during grain-filling. A possible explanation was that TNC transport to the panicles was limited by some unknown factors and TNC accumulation in the stems occurred. The increase in total dry weight during grain-filling usually showed a close correlation with grain yield[8]. Unpublished data of this laboratory found exceptions against such rule, suggesting the distribution efficiency of dry matter was important to the yield performance of rice crops. However, comparison between the two varieties (Table 3) did show a parallel relationship between dry weight increase in whole tiller after heading and panicle weight at harvest. In other words, the large-grain Mira rice possessed a pronounced higher potential in dry matter production on a per tiller basis than the medium-grain Tainung No. 67.

The net production of TNC per tiller during grain development was also significantly higher for Mira (Table 5). Difference between varieties was more pronounced in the second crop season. Those facts, along with the performances of dry matter accumulation mentioned above, clearly indicated that the productivity of single tiller was higher for Mira rice with large panicle volume. It was also suggested that yield potential of the commercial varieties was able to be increased through the improvement of genetic backgrounds.

Table 6 showed different results when productivity of the two varieties were compared on community basis. The poor performance of the large-grain Mira in the field was due mainly to the fewer panicle number per hill, or the low tillering ability. It might be possible to enhance its productivity by narrowing the planting spacing from 25×25cm to, for example, 30×15cm or even narrower. However, the responses of this variety or other large-grain rice varieties to the environmental conditions remained to studied. On the other hand, if panicle number per hill of Tainung No. 67 at heading was reduced artificially from 14-16 to 7-9, one would expect higher production per tiller of dry matter and TNC during the period of grain-filling. However, as the sink volume of this medium-grain variety was limited, significant increase in productivity could not be expected. In other words, panicle size was an important factor in determining the amount of photosynthates produced by the vegetative organs.

The amount of TNC in the panicles at harvest was about 45-90 times higher than that at heading (Table 5), indicating extremely active translocation of TNC into the panicles. When the rate was expressed as per cent of the amount at maturity, TNC deposition was generally slower than dry matter and N deposition. The results of this study were also in accordance with those of MacGregor *et al.*[5] which showed that total Kjeldahl N increased at the same rate as dry matter in barley kernels. Based on the above information, it was found that the translocation of N was faster and earlier than that of TNC, at least during the early grain-filling period.

The increase rates of major components in the large-grain Mira panicles were comparable or higher than those of the medium-grain Tainung No. 67. However, the demand of various constituents by single grain or grains per panicle of Mira rice was certainly higher than those of Tainung No. 67. In addition to total dry matter and N, Liu *et al.* [4] found the naked, largekernel (40-56mg) barley varieties Hiproly and CI-4362 required

more mineral elements for kernel development that the commercial varieties (kernel weight about 30mg). It was thus important to study the balance between supply and demand of various components in terms of obtaining high productivity of large-grain rice varieties.

In conclusion, we found that the panicle volume, or sink size, of the large-grain variety Mira was at least 30% greater than that of the commercial variety Tainung No. 67. The potential of producing dry matter and TNC and absorbing N during the period of grain-filling on a per tiller basis was also significant higher for Mira. As the tillering ability was very poor, community productivity was 50% lower than that of Tainung No. 67 in the first crop. We suggested that the productivity of commercial rice varieties could be further increased by enlarging the panicle size, mainly through increasing grain size. However, the responses of such ideal rice type to environmental factors, e. g., temperature, spacing, N requirement, etc., should be further understood.

Literature Cited

1. Chen, C. S., D. D. Huang, M. C. Shen and D. J. Liu. 1980. Carbohydrate, N, P and K status of developing rice (*Oryza sativa* L.) plants grown in the first and second crop seasons in Taiwan. J. Agri. Res. China 29 : 81-92.
2. Cock, J. H. and S. Yoshida. 1972. Accumulation of ^{14}C-labelled carbohydrate before flowering and its subsequent redistribution and respiration in the rice plant. Proc. Crop Sci. Soc. Japan 41 : 226-234.
3. Ishizuka, Y. 1969. Engineering for higher yields. *In* Physiological aspects of crop yield (J. D. Eastin, F. A. Haskins, C. Y. Sullivan and C. H. M. van Baveleds.). Amer. Soc. Agron., Madison.
4. Liu, D. J., Y. Pomeranz and G. S. Robbins. 1975. Mineral content of developing and malted barley. Cereal Chem. 52 : 678-686.
5. MacGregor, A. W., D. E. Laberge and W. O. S. Meredith. 1971. Changes in barley kernels during growth and maturation. Cereal Chem. 48 : 255-259.
6. Matsushima, S. 1975. Crop science in rice-theory of yield determination and its application. pp. 237, 255-256, 263-264. Fuji Publ. Co., Tokyo.
7. Matsushima, S. 1975. High-yielding rice cultivation. pp. . 316-317, 323-324. Univ. of Tokyo Press, Tokyo.
8. Murata, Y. and Y. Togari. 1972. Analysis of the effect of climatic factors upon the productivity of rice at different localities in Japan. Proc. Crop Sci. Soc. Japan 41 : 372-387.
9. Murata, Y. and S. Matsushima. 1975. Rice. *In* Crop physiology some case histories (L. T. Evans ed.). Cambridge Univ. Press, London.
10. Oritani, T., T. Enbutsu and R. Yoshida. 1979. Studies on nitrogen metabolism in crop plants. XVI. Changes in photosynthesis and nitrogen metabolism in relation to leaf area growth of several rice varieties. Japan J. Crop Sci. 48 : 10-16.
11. Smith, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wis. Agri. Exp. Sta. Res. Rep. 41. 11p.
12. Tanaka, A. 1961. Studies on the nutrio-physiology of leaves of rice. plant. J. Fac. Agri., Hokkaido

Univ. 51 : 449-550.

13. Wei, M. L., M. C. Shen, C. S. Chen and D. J. Liu. 1982. Physiological studies of rice tillers. I. Partition of dry matter, nitrogen and total nonstructural carbohydrates during grain-filling. Proc. Natl. Sci. Counc. ROC (A) 6 : 190-196.

14. Wu, H. P., I. Y. Liao, M. H. Chien, T. L. Lin, Y. S. Chen, Y. P. Wang, K. H. Tsai. L. K. Wu, W. I. Chang, F. H. Lin and Y. L. Wu. 1975. The investigation on the causes of low yielding in the second crop of rice. Natl. Sci. Council Monthly 3(10) : 5-39.

15. Yoshida, S. 1981. Fundamentals of rice crop science. pp. 60-62, 140, 242. International Rice Researh Institute, Los Banos, Philippines.

水稻分蘖之生理研究

II. 不同穗部容積品種的生產力比較[1]

魏夢麗　郭益全　劉大江[2]

摘　要

以大粒種水稻品種 Mira（千粒重約 37g）及中粒型水稻品種臺農67號（千粒重約 25g）爲材料，自抽穗起每週以分蘖爲單位取樣，測定源自不同節位之葉片、葉鞘、莖及穗部的乾物重，全氮及非構造性碳水化合物（TNC）含量的變化，目的在比較兩型水稻的生產能力。試驗結果發現 Mira 各器官內之全氮濃度顯著較臺農67號爲高，每一分蘖吸收的氮量亦超出臺農67號40%以上。在穀粒充實過程的淨乾物質與 TNC 生成量比較，如以單一分蘖爲單位，臺農67號分別較 Mira 少14—55%與36—39%；成熟期時，臺農67號水稻之單一穗重僅爲 Mira 之 61—74%，單一穗內 TNC 含量僅爲63—80%，顯示大粒型水稻單一分蘖的生產力較高，而推廣品種亦有再加改良的可能。由於大粒型水稻的穗數太少，故就羣落生產力而言，一期作臺農67號較 Mira 高50%，二期作則兩品種相似，即 Mira 在第二期作有較優良的表現。兩品種穗部主要成份的累積速率相當，表示絕對轉流量以大粒型品種爲高。本試驗結果建議，經由增大穀粒體積謀求稻作增產或有可能，但較大粒型水稻品種（系）對各項環境因子一諸如溫度，栽植密度、氮需要量等之反應，仍應先加以深入的瞭解。

1.臺灣省農業試驗所　研究報告第1140號。本計畫承行政院農業發展委員會補助，謹致謝意。

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中華農業研究（Jour. Agric. Res. China）33(1)：024~029（1984）

低温處理及液體培養對水稻花葯癒傷組織形成之效果[1]

蔡 新 聲　　陳 良 築[2]

摘要：水稻花葯培養前，將附有劍葉之稻穗經 10 ℃，7 天之低溫處理，可提高花葯 callus 形成率一倍，花葯接種於培養基後再行低溫處理則無效。 低溫處理花葯所得 callus ，分化之綠苗，有較多單倍體而較少雙倍體的趨勢。

液體培養不但無益於花葯 callus 之誘導，且形成之 callus 分化力極差，故不適宜水稻花葯培養。

1968年 Niizeki and Oono 首先利用水稻花葯培養誘得單倍植物體，唯因誘導花葯產生 callus 的頻率太低，限制了花葯培養技術被實際應用於育種工作上 (14,17) 。十幾年來，經由許多研究人員不斷努力的結果，已使水稻花葯培養的育種效率提高許多，如 Chen 發現單核期的水稻花葯進行培養可得最高的 callus 形成率 (2) ，Genovesi 等報導水稻花葯培養前行低溫處理，可使 callus 之誘導率提高四倍之多 (10) ，Chu 等所發表的 N_6 培養基則比其他培養基（如MS, Miller 等）更適合誘導水稻花葯產生 callus (7,12,13) 而 Chen 等所改良之 NK 培養基則又比 N_6 更優良，不但提高水稻花葯 callus 之誘導率，而且使 callus 分化綠苗之機會大爲增加 (5) 。凡此改進均已顯著提高了水稻花葯培養的育種效率，使利用水稻花葯培養技術從事育種工作趨於可能。

也有報告指出，利用液體培養技術，由於培養之花葯較易吸收養份，且可將花葯壁褐化時所產生之有毒物質洗去，所以效果較好 (11,18,21) 。本研究之目的在探討低溫處理及液體培養之效果，以供實際配合育種時之應用及參考。

材料與方法

一、花葯的消毒及接種

花葯培養所用的材料爲粳稻臺農67號品種。將劍葉葉耳距下位葉葉耳約 5 cm 之稻穗連同劍葉取下；低溫處理稻穗之方式係以濕潤之棉花包裹葉鞘基部，裝入塑膠袋中，置於恆溫 10°C 之冰箱中 7 天。預備採取花葯之材料，先以 70% 酒精擦拭葉鞘外表後，取出稻穗浸漬於 70%酒精中30 sec 再以 0.5% 次氯酸鈉消毒 3 min 後，以無菌水冲洗 3 ~ 4 次，將屬於單核期之花葯以 Chen 等 (3) 所述之法，接種於培養基上。

二、使用培養基之組成及種類

（一）低溫處理試驗之培養基：以無機鹽減半（Na-Fe-EDTA 除外）之基本 MS（1962）配方，配合4 mg/l NAA, 2 mg/l Kinetin，6 %蔗糖及0.8% phytagar，做爲誘導水稻花葯 callus 形成之培養基。

1.臺灣省農業試驗所　研究報告第1141號。本研究承國科會之補助（NCS 72-0409-B055-01），特此致謝。
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(二) 液體培養試驗之培養基：含有 Chu *et al.*[7] 無機鹽類，MS 之有機物，配合 4 mg/l NAA, 2mg/l Kinetin 及 6 %蔗糖之改良式 NK 培養基[5]為本試驗之基本培養基。固體培養基則以 0.8% phytagar 凝固之，並以 25×120 mm 之試管盛裝，每支試管含 10 ml 之培養基，液體培養基則以 10 ml 之量，裝於 125 ml 三角瓶中。液體培養初期四週為靜止培養，callus 開始形成後再行 60 rpm 之往復式震盪培養。

花葯培養於 26±1 °C 恆溫、黑暗下，進行 callus 之誘導。誘導 callus 分化植物體之方式，則取10天日齡之 callus 培養於含有 1 mg/l NAA、4 mg/l Kinetin、170 mg/l NaH_2PO_4、40 mg/l adenine sulfate 及 3% 蔗糖之基本 MS 培養基上，在26±1°C 恆溫、光照1,500 lux 光期16小時的環境下培養。

結果與討論

一、低温處理

表 1 顯示，附有劍葉之稻穗於 10 °C 低溫下處理 7 天後之花葯，其 callus 形成率高達 41.9%，較不經低溫處理對照組 (CK-1) 之 21.2% 高出甚多，幾達一倍左右；而將花葯接種於培養基後再行低溫處理者，其 callus 誘導率為 26.4%，雖略高於未處理對照 (CK-2) 之 22.4%，但統計分析並無顯著差異。由此結果可知花葯接種前，將附有劍葉之稻穗以 10 °C 低溫經 7 天之處理，對提高花葯 callus 之形成率非常有效。

表1. 低溫處理水稻花葯對 callus 形成之效果

Table 1. Effect of low temperature treatment on callus induction in rice anther culture (cv. Tainung 67).

Material	Treatment	No. of anthers cultured	No. of anthers forming callus	Percent of anthers forming callus
Panicle with flag leaf	10°C for 1 week	1,094	452	41.9[a]
	CK-1	93	202	21.2[b]
Anthers on culture medium	10°C for 1 week	667	179	26.4[b]
	CK-2	700	151	22.4[b]

Basal medium : Murashige and Skoog (1962) organic salts, ½ strength of MS mineral salts (except Na-Fe-DETA), 6% sucrose, 4mg/l NAA and 2mg/l kinetin.

將低溫處理及未經低溫處理所誘得之 callus，分別繼代培養於分化培養基以誘導植物體形成，其形成植物之染色體倍數性如表 2 所示，經低溫處理所誘得之花粉來源植物體，單倍體佔88%，雙倍體及多倍體僅各佔 7 %及 5 %，而未經低溫處理者，雙倍體可達38%，這種結果顯示低溫處理可使水稻花葯培養誘得 callus 形成之植物體趨向單倍體。

表2. 低溫處理水稻花葯對 callus 分化所得植物染色體倍數的效果

Table 2. Effect of cold shock on the chromosome distribution of anther-derived plants in rice anther culture (cv. Tainung 67).

Treatment	No. of anther-derived plants investigated	% of ploidy level		
		N	2N	polyN
CK	48	58	38	4
Cold shock*	43	88	7	5

*Unemerged panicles with flag leaf were kept under 10°C for 1 week before anther culture.

低溫處理花葯可促進單倍胚狀體形成的效果，早於1973年 Nitsch and Norreel 就在曼陀蘿的花葯培養中發現，氏等認爲低溫處理，使花粉第一次有絲分裂產生大小相等的兩個細胞核，只有這種花粉將來才能形成胚狀體(15)。Vasil 解釋爲低溫可引起花葯內代謝活性的減低，而使大量花粉粒停留於轉換成 sporophytic 發育所需之時期(19)。Duncan and Heberle 則認爲低溫延遲了花粉粒劣變的過程，使較多花粉粒開始新的細胞分裂，而導致胚狀體形成(9)。

低溫處理水稻花葯的效果，許多學者均曾報導，其效果依處理溫度之高低及時間長短而有顯著之不同，一般介於 6—13 °C 之低溫處理均可提高 callus誘導率(1,10,20)。太低的溫度如3—4 °C以下，處理時間極不易控制(8)。Genovesi and Magill 認爲較溫和之低溫如 10—13 °C 處理10—14天效果最好，可增加 callus 的誘導率4倍之多(10)；超過15天的處理會造成誘得之 callus 較難分化，且形成較多的白苗(6,10,22)。本研究發現將附有劍葉，穗基包以濕潤棉花，置於塑膠袋內再經8—10°C之低溫處理，可增加callus 之誘導率一倍；將花葯切取後放置培養基上再行低溫處理之效果則不顯著，和前人之結果十分相似。Chen 等(5)檢討培養基的效果發現，改良 NK 培養基亦可使水稻花葯 callus 之誘導率提高一倍，是否改良NK培養基及低溫處理花葯二者配合使用，對 callus 之誘導具有累加效果，頗值研究。在植物體誘導方面，低溫處理似乎增加了單倍體形成的比例，這種使自然倍加二倍體植物形成機會減少的情形，十分不利於實際育種，所以尋找一種可增加 callus 誘導率，而又不會減少自然倍加二倍體形成之花葯低溫處理方式，頗值進一步探討。

二、液體培養

利用改良後之固體或液體 NK 培養基，及將植物荷爾蒙或蔗糖用量減半之液體培養基，測定其對水稻花葯 callus誘導之效果，結果如表3顯示，含有相同植物荷爾蒙及蔗糖用量之固體與液體培養基，對 callus之誘導有極大差異；液體培養者可見之callus數目爲56.9%，而固體培養者則僅39.1%，這種結果從數字上看好像液體培養之效果優於固體培養，其實固體培養時，一個花葯可長出數個 callus，不同花粉來源之 callus 在短時間內即因生長混合而無法分辨，故極難以 callus 爲單位計算。統計上若改以 callus 形成之數目計算，則固體培養的實際效果將超出液體培養甚多。至於同爲液體培養，將荷爾蒙 NAA 及 Kinetin 或蔗糖濃度減半，對 callus 形成之影響可由表3得知，蔗糖濃度減半者其 callus 形成率爲53.3%與不減半者之56.9%幾無差異，而 NAA 及 Kinetin 減半者，其 callus 形成率 43.1% 則較不減半者更低，這種結果說明在液體培養之環境下，植物荷爾蒙 NAA 4 mg/l 與 Kinetin 2 mg/l 仍較適合，蔗糖則影響不大。

表3. 固體或液體培養基對水稻花葯 callus 形成之效果

Table 3. The effects of liquid and solid media on callus induction in rice anther culture (cv. Tainung 67)

Treatment*	No. of anthers cultured (A)	No. of visible callus produced (B)	(B)/(A) —%—
Solid-NK1	1,571	684**	39.1
Liquid-NK1	1,200	683	56.9
Liquid-NK2	840	362	43.1
Liquid-NK3	960	512	53.3

*Media components : NK1 : Chu *et al.* (1975) inorganic salts, MS organic compound, 6% sucrose, 4 mg/l NAA and 2mg/l kinetin.

NK2 : Same as NK1 except concentrations of NAA and kinetin were reduced to 2mg/l and 1mg/l, respectively.

NK3 : Same are NK1 except sucrose concentration was reduced to 3%.

**No. of anthers producing callus.

在不同培養基成份下誘得之 callus，分別移至分化培養基上誘導植物體形成，結果如表 4 顯示，各種液體培養基所產生 callus 分化綠苗之能力幾近於零。大部分 callus 都在移至分化培養基後二、三週內死亡，少部分 callus 分化為不正常芽體及根。而由固體培養基所形成之 callus 則有25%分化為綠苗，5 %分化為白苗，25%分化為不正常芽及根。綜合表3 、4 之結果可知液體培養水稻花葯，不適用於以產生綠苗為主要目標的育種工作，除非有抑制 callus 褐化及促進分化綠苗的技術突破，否則仍以固體培養為佳。

表4. 水稻花葯 callus 之誘導培養基對 callus 分化能力之影響

Table 4. Influence of different callus-induction media on the differentiation ability of callus in rice anther culture (cv. Tainung 67).

Callus induction media*	No. of callus cultured	No. of callus turning brown	No. of callus producing		
			Green plants	Albino plants	Abnormal shoot and root
Solid-NK1	80	36(45)**	20(25)	4(5)	20(25)
Liquid-NK1	80	64(80)	0	0	16(20)
Liquid-NK2	80	62(77)	2(3)	2(3)	14(17)
Liquid-NK3	80	50(63)	0	0	30(37)

*Media components : same as Table 1.

**( ) = percent

雜種水稻花葯培養產生 callus 時，每個花葯能自不同的小胞子分裂產生多個 callus，這些不同小胞子來源的callus, 含有不同的基因型，若能將此等不同基因型之 callus 均予以誘導分化成植物體，則少量的花葯培養即可產生各種不同基因型之後代，然因固體培養花葯佔的空間有限，而使 callus 形成後擠在一起，造成競爭而失去許多優良基因型，液體培養可克服上述缺點, Vasil [19] 認為固體培養易受老化花葯壁釋出的抑制物所影響，因此建議培養基中添加活性碳或利用液體培養可得較好的結果，Nitsch [16] 也建議液體培養有利於胚之發育。但本研究顯示水稻花葯行液體培養，不但對callus 的誘導沒有幫助，且使誘得之 callus 分化綠苗之機會大為降低，因此水稻花葯似不宜利用液體培養，此結論與前人之研究結果極不一致，可能為作物不同或受其它因素之影響，有待進一步探討。

引用文獻

1. Chaleff, R. S. and A. Stolarz. 1981. Factors influencing the frequency of callus formation among cultured rice (*Oryza sativa*) anthers. Physiol. Plant. 51 : 201-206.
2. Chen, C. C. 1977. In vitro development of plants for microspores of rice. In Vitro 13 (8) : 484-489.
3. Chen, C. C. and C. M. Chen. 1979. A. method for anther culture of rice. TCA Manual 5(2) : 1051-1053.
4. Chen, C. C. 1982. Toward utilization of anther culture in rice breeding. In : Proceeding of a Symposium on Plant Breeding (Ed. by S. C. Hsieh & D. J. Liu) pp. 81-87. Agri. Assoc. of China. Taiwan, ROC.
5. Chen, L. J., P. C. Lai, C. H. Liao and H. S. Tsay. 1982. Medium evaluation for rice anther culture. In : Proc. 5th Intl. Cong. Plant Tissue and Cell Culture. (Ed. by A. Fujiwara) pp. 551-552. The Jap. Association for Plant Tissue Culture. Tokyo.
6. Chen, Y., Q. X. Zuo, H. Y. Li and R. D. Qu. 1982. Plant regeneration from isolated rice pollen

culture and some factors affecting induction frequency. In : Proc. 5th Intl. Cong. Plant Tissue and Cell Culture. (Ed. by A. Fujiwara) pp. 559-560. The Jap. Association for Plant, Tissue Culture. Tokyo.

7. Chu, C. C., C. C. Wang, C. S. Sun, C. Hsu, K. C. Yin, C. Y. Chu and F. Y. Bi. 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sinica 17 : 657-668.
8. Cornejo-Martin, M. J. and E. Primo-Millo. 1981. Anther and pollen grain culture of rice (*Oryza sativa* L.). Euphytica 30 : 541-546.
9. Duncan, E. J. and E. Heberle. 1976. Effect of temperature shock on nuclear phenomena in microspores of *Nicotiana tobacum* and consequently on plantlet production. Protoplasma 90 : 173-177.
10. Genovesi, A. D. and C. W. Magill. 1979. Improved rate of callus and green plant production from rice anther culture following cold shock. Crop Sci. 19 : 662-664.
11. Heberle, E. and J. Reinert. 1979. Androgenesis in isolated pollen cultures of *Nicotiana tobacum* : Dependence upon pollen development. Protoplasma 99 : 237-245.
12. Miller, C. O. 1963. Kinetin and kinetin-like compounds. Modern Meth. Plant Anal. 6 : 194-202.
13. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.
14. Niizeki, H. and K. Oono. 1968. Induction of haploid rice plant from anther culture. Proc. Jpn. Acad. 44 : 554-557.
15. Nitsch, C. and B. Norreel. 1973. Effect d'un choc thermique sur le pouvoir embryogene du pollen de *Datura innoxia* cultuve dans l'anthere ou isole de l'anthere. C. R. Acad. Cci. D. 276 : 303-306.
16. Nitsch, C. 1981. Production of isogenic lines : Basic technical aspects of androgenesis. In : Plant tissue culture, methods and application in agriculture. (Ed. by T. A. Thorpe) pp. 241-252. Academic Press.
17. Oono, K. 1981. In vitro methods applied to rice. In : Plant tissue culture, methods and application in agriculture. (Ed. by T. A. Thorpe) pp. 273-289. Academic Press.
18. Sunderland, N. 1978. Strategies in the improvement of yields in anther culture. In : Proc. Symp. Plant Tissue Culture. pp. 65-86. Peking. Science Press.
19. Vasil, I. K. 1980. Androgenetic haploid. In : Perspectives in plant cell and tissue culture. (Ed. by G. H. Bourne and J. F. Danielli) pp. 195-223. Academic Press.
20. Wang, C. C., C. S. Sun and Z. C. Chu. 1974. On the conditions for the induction of rice pollen plantlets and certain factors affecting the frequency of induction. Acto Bot. Sinica 16 : 43-53.
21. Wernicke, W. and H. W. Kohlenbach. 1976. Investigations on liquid culture medium as a means of anther culture in *Nicotiana*. Z. Pflanzenphysiol. 79 : 189-198.
22. Zapata, F. J., L. B. Torrizo, R. O. Ramero and M. S. Alejar. 1982. Androgenesis in *Oryza sativa*. In : Proc. 5th Intl. Cong. Plant Tissue and Cell Culture. (Ed. by A. Fujiwara) pp. 531-532. The Jap. Assiciation for Plant Tissue Culture. Tokyo.

The Effects of Cold Shock and Liquid Medium on Callus Formation in Rice Anther Culture[1]

H. S. Tsay and L. J. Chen[2]

Summary

Rice culm with flag leaf was pretreated by low temperature at 10°C for 7 days. Anthers taken from these pretreated flowers were cultured on solid medium. It was found that cold-shocked anthers produced more callus (2-fold) than those without pre-treatment. The effect was not significant when pretreatment was done after anthers have been cultured on medium. Callus initiated from cold-shocked anthers produced more haploid plants and less diploid plants than the callus initiated from anthers without pretreatment.

Due to the low differentiation ability of callus initiated from anthers cultured in the liquid medium, it is considered that liquid suspension culture is not suitable for rice anther culture.

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1. Contribution No. 1141 from Taiwan Agricultural Research Institute. This study was supported by a grant (NSC72-0409-BO55-01) from National Science Council, Republic of China.

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中華農業研究 (Jour. Agric. Res. China) 33(1)：030～037 (1984)

油瓜 (*Cucurbita foetidissima* HBK) 引種試驗及其種子與塊根成分分析[1]

韓青梅 莊周瑞 胡敏夫 邱善美[2]

摘要：本文目的在探討新引進之油瓜 (Buffalo gourd) 品系在中部地區之適應性，同時分析其種子及塊根等之主要成分。

經三年 (1979—1981) 之品系觀察試驗結果顯示，油瓜可在本省中部地區生長，蔓葉滋生迅速而旺盛，但由於氣候多雨潮溼，受多種病蟲所侵襲。每株果數太少 (1.2—6.8果／株；平均僅收穫2.8果／株) 及每果種子不多 (72—257粒／果)，爲其缺點。

種子之油脂及蛋白質含量分別爲33.5～37.5% 及 24.4～25.2%，根部澱粉含量約爲56～73%之間，概依供試品系之不同有所差異。

根部之適當收穫，宜在地上部枯萎後即行收穫，並應立即抽取澱粉。留置土中過久或貯藏日數太長都使澱粉抽取率降低。

油瓜 (Buffalo gourd, *Cucurbita foetidissima* HBK) 原產於美國西南部及墨西哥北部，爲蔓生、葡伏性、耐旱，生育迅速及適應性頗强的一種多年生野生植物。居住於當地之印第安人取其種子，經磨碎後作成濃粥，或經炒 (煮) 熟後供食用，或以磨碎之種子藉熱水抽取其油分，供爲化粧品，纖維則用爲淸潔劑，根部爲醫藥用 (6,7,8)。種子含蛋白質及油分，根含澱粉質頗豐，是一種油脂、蛋白質及澱粉兼用植物，極具開發發展潛力 (6,7,8,9,10,11)。

民國68年行政院農業發展委員會屈技正先澤自美國亞利桑那州引入7個油瓜品 (種) 系，撥交省農試所、臺南及高雄區農業改良場，進行品系觀察試驗，以探討其對本省之適應性；本所經三年 (1979—1981) 觀察試驗，並自第2年 (1980) 起分析種子及塊根成分，同時探討塊根之收穫期和貯藏日數對澱粉含量之影響，所獲結果可供爲本省山坡地栽培新興作物之參考。

材料與方法

一、試驗材料計下列7個品 (種) 系：

1. 140×142
2. 142×158
3. (140×156)×300
4. 158×142
5. 162×142
6. 185×142
7. 250×300

二、試驗方法：

1.品 (種) 系觀察試驗

順序排列、不設重複、作畦栽培、畦寬 3m、長 25m、穴播、穴距 2m，以三角形等距離種植，

1.臺灣省農業試驗所 研究報告第 1142 號。承行政院農業發展委員會補助經費，屈技正先澤的鼓勵及張義璋先生分離病原菌，謹此一併致謝。

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播種期第一年（1979）於4月中旬，而後之2年（1980—1981）概在5月上旬播種，每穴播5粒種子，每品系植15穴（後2年每品系植30穴），待苗高 15 cm 左右時行間拔，每穴留苗1株。生育期間計施肥2次，每次每穴各施硫銨4克，過磷酸石灰6克及氯化鉀2克。視土壤乾燥程度，行溝灌溉1—2次。調查項目包括雌雄花顯花期，一株收穫果數，鮮果重及種子性狀等。

2.種子及塊根成分分析

（1）粗蛋白質之測定，採用 Kjeldahl 法[1,3]。

（2）粗脂肪含量之測定，應用 Soxhlet 法[1,3]。

（3）粗澱粉含量之測定，利用 Somogyi 法[1]。

（4）纖維之測定採用酸鹼溶解法[2]。至於水分及灰分之測定依李秀等著「食品分析與檢驗」所列舉方法進行[1]。

3.塊根採收期對其成分之影響

以品系158×142及162×142爲試材，於地上部蔓葉枯萎後行第一次（1981年11月20日）採收，而後分別於同年12月15日及1982年1月5日和塊根萌芽後各採收一次。每次約 1kg，攜回室內洗淨後陰乾，於翌日進行分析，採用方法同方法2。

4.塊根貯存日數對其成分之影響

使用材料亦爲158×142及162×142等2個品系。將同日（1981年11月20日）採收之塊根，不經水洗，即貯存於網室之水泥地面上，經5日及8日後，分別取樣分析，方法同2。

結果與討論

一、植物形態

油瓜爲蔓生植物、蔓粗大、呈圓筒形，由節及節間構成。蔓平伏地面、淡綠色、具毛茸、易折斷、不耐朝濕，浸水後容易導至腐爛。蔓節可形成不定根（adventitious root）。葉濶大呈心臟形、葉尖尖銳、淡綠色、葉緣無缺刻或具淺缺刻（依供試品系而異），葉面粗糙具毛茸，葉柄頗長，著生於蔓節上（圖1）。

花爲單性花（unisexual flower），黃色，合瓣如漏斗，雌雄同株（monoecious），或全株均開雌花，如品系(140×156）×300。雌雄花均於早上開花，午後花謝，果實圓形而堅硬，不易敲破，直徑 4—7 cm。未熟果實呈綠色，成熟後呈橙黃色，表面具紋路，頗爲美觀（圖2）。果實內儲種子70—200 粒不等。種子白色或呈黃褐色，長橢圓形，較南瓜種子爲小（圖 3）。種子百粒重約 4—6.9 g（圖3）。根膨大，具少數鬚根、淡褐色（圖4）。

不論是自蔓節所發生的不定根或發自種子之原始根，均具極强之再生力，俟地上部於冬季枯萎後，此等留置於土壤中之根，可重新萌發新芽，形成新植株，繼續生長。

二、植物性狀調查

表1及表2係就3年來品系觀察各供試品系中，隨機選出生育正常之單株5株，於開花期調查其雌雄花始花期及花數之結果。據表1，雄花之始花期約在播種後之 82.2天（250×300）至 93.6天（185×142）之間，而品系（140×156）×300未出現雄性花，據 Bemis 等[6,7,8]之報告，雄性花出之現受一對顯性因子所控制。每株之雄花數以品系185×142最少（2.8朵），142×158最多（7.2朵）。

雌花之始花期概較雄花爲晚約10餘日，每株之雌花數，除品系158×142外，其他品系均較雄花數爲多，尤以162×142最爲突出，每株雌花數達11.2朵（表1）。

不同月份雌花之開花數，隨供試品系之不同而異，一般來說，品系158×142之盛花期在7月，自9月起即不再有雌性花顯現，花期頗短；其他品系之盛花期在8月，至9月仍有雌花顯現，花期顯較158×142爲長（表2）。但此時所開之花，由於氣溫逐漸降低，所生成果實之果型較小，種子充實度較差。

Fig. 1. Male flowers and vigorous vegetative growth of buffalo gourd at the experimantal farm of TARI.

Fig. 2. Fruits of buffalo gourd.

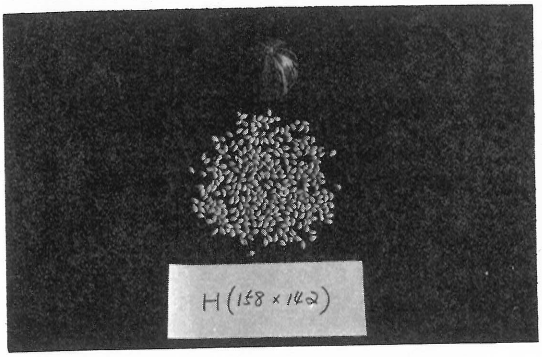

Fig. 3. Seeds of buffalo gourd.

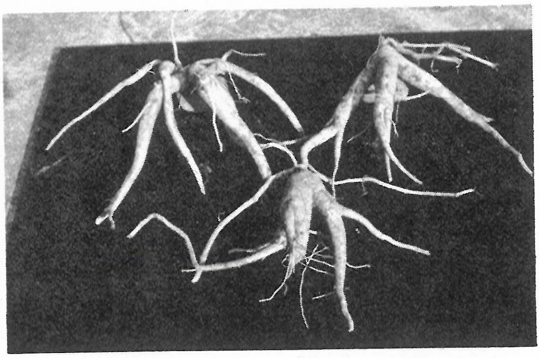

Fig. 4. Root system buffalo gourd of 7 months of growth.

Table 1. Timing of flowering and number of flowers per plant of different buffalo gourd strains (1979–1981).

Strain	Days from sowing to 1st male flowering	Days from sowing to 1st female flowering	No. of staminate flowers/plant	No. of pistillate flowers/plant
140×142	88.7	101.4	6.7	7.5
142×158	86.3	98.6	7.2	9.4
(140×156)×300	—	94.5	—	6.8
158×142	83.5	99.6	5.6	3.9
162×142	91.4	103.4	5.4	11.2
185×142	93.6	104.7	2.8	3.2
250×300	82.2	96.4	3.1	3.6
Average	87.6	99.8	5.1	6.5

Table 2. Monthly distribution of the number of female flowers of different buffalo gourd strains (1979-1981).

Strain	Number of pistillate flowers per plant				
	July	Aug.	Sept.	Oct.	Total
140×142	0	4.3	2,7	0.5	7.5
142×158	1.2	6.8	1.2	0.2	9.4
(140×156)×300	0.8	4.3	1.7	0	6.8
158×142	3.2	0.7	0	0	11.9
162×142	0.8	5.6	3.8	1.0	11.2
185×142	0	2.6	0.6	0	3.2
250×300	0	2.4	1.2	0	3.6
Total	6.0	26.7	11.2	1.7	

雌花之始花期概較雄花爲晚約10餘日，每株之雌花數，除品系158×142外，其他品系均較雄花數爲多，尤以162×142最爲突，每株雌花數達11.2朵（表1）。

不同月份雌花之開花數，隨供試品系之不同而異，一般來說，品系158×142之盛花期在7月，自9月起即不再有雌性花顯現，花期頗短；其他品系之盛花期在8月，至9月仍有雌花顯現，花期顯較158×142爲長（表2）。但此時所開之花，由於氣溫逐漸降低，所生成果實之果型較小，種子充實度較差。

各供試品系之果實性狀如表3，不論係果實鮮重，果實大小，每果種子數等，概以品系162×142及158×142較爲優異，而以（140×156）×300最差。

Table 3. Fruit characteristics of different strains of buffalo group (1979-1981).

Strain	Fresh Wt. per fruit (%)	Diameter of fruit (cm)	No. of fruit/plant	No. of seed/fruit	% of filled seed	100 seed Wt. (g)
140×142	106.6	4.75	3.5	183.5	85.8	4.9
142×158	110.2	5.04	2.9	205.6	86.2	5.1
(140×156)×300	74.4	3.87	1.7	71.9	82.4	4.1
158×142	106.2	5.85	1.2	202.6	88.1	6.1
162×142	110.7	5.87	6.8	257.3	82.5	5.1
185×142	118.4	5.84	1.7	190.9	84.2	4.3
250×300	97.1	4.80	1.7	200.2	81.6	4.3
Average	103.4	5.15	2.8	187.4	84.4	4.8

各供試品系之塊根性狀，經調查結果如表4。一株塊根平均鮮重（生育期約7個月），以品系250×300最重，超過 1kg，次爲 162×42 及 158×142，各爲 874 g 及 826 g，而以（140×156）×300 最少，僅 664 g。

Table 4. Root characteristics of different strains of buffalo gourd (1980)[a]

Strain	Root diameter (cm)	Length of root (cm)	Fresh Wt. (g/plant)
140×142	6.7	39.6	684
142×158	4.5	38.0	736
(140×150)×300	7.7	37.8	664
158×142	4.8	48.0	826
162×142	6.0	38.6	874
185×142	5.7	42.4	806
250×300	7.4	40.6	1,276
Average	6.1	40.7	838

[a] About 7-month-old.

三、病蟲害調查

（一）病害：

1.凋萎病：發生於發芽後70天左右，導致根部腐爛，植株枯萎，情況嚴重時可使全區植株毀滅，尤其在連續陰雨後之土壤，溼度高時，發生最為嚴重，該病害經本所植物病理系自其病根分離得2種病原菌，一為猝倒病菌（Pythium sp. ）另一為腰折病菌（Rhizoctonic sp. ）。

2.蔓割病：於開花結果期較為嚴重，尤其在潮溼環境下最易發生，致使感染株蔓枯萎，果實落果等現象。

3.其他病害：在植株生育初期，尚發現露菌病，生育後期受白粉病之侵害，但在為害程度上遠較凋萎病及蔓割病為輕。

（二）蟲害：

1.瓜蠅：為最嚴重的一種蟲害，如能有效控制，即可增加每株收穫果數。

2.其他蟲害：有蚜蟲及線蟲，前者發生於種子萌芽後，具4—5葉片時較嚴重。後者在本所試區未發現，屏東試區頗為嚴重[5]。

四、種子及塊根成分分析：

種子成分分析係以品系162×142及158×142為供試材料，所獲結果如表5。各品系之塊根成分分析結果列如表6。

由表5知，兩品系種子之粗蛋白質、粗脂肪、粗纖維及灰分含量略有差異。粗蛋白質及粗纖維之含量以品系162×142者略高；粗脂肪及灰分之含量則以品系158×142者為高。而兩品系種子各成分之平均含量為粗蛋白質24.80%、粗脂肪35.46%、粗纖維7.62%、灰分3.60%。此數據與 Berry等[10]報告之油瓜種子含蛋白質32.9%、粗脂肪33.0%、粗纖維15.1%、灰分 3.1%之分析結果互有消長，尤以粗蛋白質及粗纖維為然，此當為栽培環境及供試品種有異之故。

7個品系塊根之澱粉含量相當高，變域為56—73%，平均達64%，其中以140×142及158×142兩品系較高，其澱粉含量分別為73%與71%，而以（140×150） ×300 品系最低，僅56%。粗脂肪及灰分之含量，供試7品系之平均分別為4.28%與4.19%，不僅含量相近，兩者之變域亦十分類似。蛋白質含量平均為6.93%，除142×158品系較高達10%外，餘均在5—7%之間。粗纖維含量之平均則為10.26%，變域介乎6.87—16.67%。

比較油瓜種子與塊根之粗脂肪及粗蛋白質含量（表5及表6）。互知油瓜種子之粗脂肪與粗蛋白

質含量約爲塊根者之9—4倍。同時數據亦顯示油瓜種子之粗脂肪含量略高粗蛋白質含量，而塊根者反是。

Table 5. Chemical composition of seeds of two buffalo group strains (dry basis).

Strain	Crude fat (%)	Crude protein (%)	Fiber (%)	Ash (%)
162×142	33.45	25.20	8.43	3.55
158×142	37.46	24.40	6.79	3.85
Average	35.46	24.80	7.62	3.60

Table 6. Chemical composition of roots buffalo gourd strains (dry basis).

Strain	Crude fat (%)	Crude protein (%)	Starch (%)	Fiber (%)	Ash (%)
140×142	4.03	5.83	73.03	7.23	4.23
142×158	4.86	10.00	62.10	9.13	3.43
(140×156)×300	4.74	7.13	56.34	16.67	5.54
158×142	3.20	6.06	71.01	6.87	3.37
162×142	4.94	6.43	65.05	8.39	3.38
185×142	4.66	7.66	63.44	11.27	4.39
250×300	3.57	5.40	57.62	12.27	4.97
Average	4.28	6.93	64.08	10.26	4.19

Note : See table 4.

五、油瓜塊根採收期及貯存日數對其成分之影響

亦以品系162×142及158×142爲供試材料，所獲結果分別列如表7及表8。自表7獲知2個品系塊根之平均澱粉含量以11月20日所採收者最高（64.1%），此時恰爲地上部蔓葉枯萎後的第6天，嗣後逐漸下降。蛋白質含量則依採收期的先後逐次增加，顯示在臺中地區之自然環境下，留置於土中之塊根時期均在作萌芽的準備，致使塊根內所儲蓄之澱粉量呈不穩定狀態，因此欲獲得較高之澱粉收量，適期的收穫至爲重要。

Table 7. Effect of harvesting time on the composition of roots of buffalo gourd (average, dry basis).

Harvesting date[a]	Crude fat (%)	Crude protein (%)	Crude starch (%)	Fiber (%)	Ash (%)
Nov. 20, 1981	4.1	6.9	64.1	10.2	4.2
Dec. 15, 1981	4.3	7.7	59.1	10.9	4.3
Jan. 5, 1982	5.1	8.6	55.2	10.2	4.0
Jan. 24. 1982 (5-day after sprouting.)	4.3	11.2	48.5	11.5	5.7

[a]Planting date was May 1th 1981.

Table 8. Effect of storage period on the composition of roots of buffalo gourd (average, dry basis).

Days of storage[a]	Crude fat (%)	Crude protein (%)	Crude starch (%)	Fiber (%)	Ash (%)
5	5.9	7.4	53.3	11.4	3.9
8	6.5	9.5	51.0	14.1	4.7
CK	4.1	6.9	64.1	10.2	4.2

[a] Harvesting date was Nov. 20, 1981.

類似的結果也發生於收穫後貯存處理試驗（表8），如以當日收穫之塊根所含澱粉量爲對照，則在暴露之情況下貯存5天後，其澱粉含量約損失4.8%，經8天後損失 13.1%，顯示貯存日數愈久，澱粉之收取量愈少，損失量愈大，在加工處理應加注意。

綜合本所三年之觀察試驗及參照臺南[4]與高雄[5]二農業改良場之試驗結果顯示，油瓜可在本省中南部地區生長，蔓葉迅速滋生而旺盛，惟由於氣候多雨潮溼，病蟲害侵害機會較多，在耕作上受到自然條件的限制。每株收穫果數，除本所在每次之試驗區中，依品系（種）別收穫少數果實外，其他試驗處所均無所獲。

據 Bemis 等[6,7,8]之報告，油瓜果實可連續收穫，每株約可收穫200個以上。本所所作試驗結果顯示，在7個供試品系中，每株收穫果數最多僅6.8果（162×142），最小者僅有1.2果（158×142），主要原因可能係由於環境因素關係及病蟲侵害所導致之結果，然自每期作每單株具有的雌花數言，欲達到每株結果數在200個以上，似無可能。其次，每果種子數較其原產地爲少，也是事實。

在本省中部地區，油瓜植株生育至10月下旬至11月上中旬時，地上部之蔓葉即開始枯萎，葉色自淡綠色轉變爲淡褐色，葉面草質化，至11月中下旬全部枯萎。此時留置於土壤中之塊根，通常於12月底或1月間即行萌芽，但隨後雨季來臨，再生新蔓遭受侵害而枯萎，欲以宿根連續繁殖，頗爲困難，如爲排水不良之土地，所受影響更大。

參考文獻

1. 李秀・賴滋漢・1979・食品分析與檢驗。精華出版社 p. 161-232.
2. 谷雲川・邱俊雄・1972・臺灣主要竹材纖維形態及化學組成試驗。臺灣省林業試驗所合作報告第20號
3. 何芳陔・張爲憲・丁一倪・1971生物化學實驗。環球書社 p. 243-247.
4. 臺南區農業改良場・1980・油瓜品種觀察試驗。
5. 高雄區農業改良場・1980・油瓜觀察試驗初期報告。
6. Bemis, W. P., J. W. Berry and C. W. Weder. 1977. The buffalo gourd-a potential arid land Crop. New Agri. Crop. AASS Selected Symposium, Westview Press, 5500 Central Avenue, Boulder, Colorado. pp. 65-85.
7. Bemis, W. P., L. D. Curtis, C. W. Weder and J. Berry. 1978. The feral buffalo gourd, *Cucurbita foetidissima*. Econ. Bot. 32(2) : 87-95.
8. Bemis, W. P., J. W. Berry, C. W. Weber and T. W. Whitaker. 1978. The buffalo gourd : a new potential horticultural crop. Hortsci. 13(3) : 235-240.
9. Ba-Amer, M. A. and W. P. Bemis. 1968. Fruit and seed development in *Cucurbita foetidissima*. Econ. Bot. 22 : 297-299.
10. Berry, J. W., C. W. Weber, M. L. Dreher and W. P. Bemis. 1976. Chemical composition of buffalo gourd, a potential food source. Food Sci. 41 : 465-466.
11. Jacks, T. J., T. P. Hensarling and L. Y. Yatsu. 1972. Cucurbit seed. I. Characterizations and uses of oils and proteins. Econ. Bot. 26(2) : 135-141.

Studies on the Flowering Characteristics and Chemical Analysis of *Cucurbita foetidissima* HBK[1]

C. M. Han, J. R. Juang, M. F. Hu and S. M. Chiu[2]

Summary

This experiment was carried out from 1979 to 1981 at the experimental farm of TARI to study the adaptation of newly-introduced strains of buffalo gourd (*Cucurbita foetidissima* HBK) and the chemical composition of seeds and roots. Vigorous vegetative growth was obsevred on all strains. However, serious damage by Fusarium wilt, powdery mildew and other diseases occurred during the growth period especially in the raining season. Melon fly was the major insect pest which caused severe damage on the fruits. Only a limited number of fruits was able to be harvested (average at 2.8 fruits per plant). The number of seeds was 72-257 per fruit. The crude fat and crude protein content of seeds were 33.5-37.5% and 24.4-25.2%, respectively. The starch content of roots (7-month) was 56-73%, depending on the strain of buffalo gourd tested. The best harvest time of roots was when withering occurred on the above-ground portion of the plants. Delay of harvest would cause an increase of fat and protein concentrations but a decrease in starch. Similar results were found if the roots had been stored for 5 to 8 days.

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Jour. Agric. Res. China (中華農業研究) 33(1) : 038~043 (1984)

Effect of Plant Density on the Yield of Taro in Paddy Field[1]

Tsung-Dao Liou[2]

Abstract : Taro, cv. Betelnut, were planted in paddy fields in 1979 and 1980 showed that the higher the plant density, the higher the plant and yield. However, size, weight and number of main corm or cormels produced on each plant were negatively correlated to the plant density. The highest main corm yield was recorded in the plot with a spacing of 60 × 20 cm (83,300 plants/ha) of 36 tons/ha and 55 tons/ha and the lowest in the spacing of 60 × 60 cm (26,600 plants/ha) of 17 tons/ha and 21 tons/ha respectively in 1979 and 1980. Total yield (main corm + cormels) also showed the highest in the spacing of 60 × 20 cm of 43 tons/ha and 63 tons/ha and the lowest in the spacing of 60 × 60 cm of 22 tons/ha and 26 tons/ha respectively in 1979 and 1980. This information is valuable for the farmers to cultivate taro in the paddy fields.

Taro (*Colocasia esculenta* (L.) Schott) is a herbaceous perennial of the Arum family. It probably originated in Northern India and is a crop widely cultivated in most tropical and subtropical areas of the world[(3)]. It contains lots of carbohydrates, minerals and vitamins and is widely used, either boiled or pasted, as the staple food of the people in the tropical and subtropical Pacific Basins[(11,12,13)]. In Taiwan, it is commonly used in the preparation of ice cream and cake[(1,6,14)]. It can also be used to make taro chips. The housewives use main corm, cormels, young leaves and petioles as vegetables. The farmers like to grow it because it is easily to be cultivated under high temperature and resistance to waterlogging during tropical monsoon season.

Taro is mainly grown in paddy fields in central and southern parts of Taiwan. However, scattered plantings also were observed in the upland or slope land[(15)]. The acreage of taro cultivation was increasing year by year. Up to 1981, 4,407 hectares were planted[(2)]. In general, the farmers favored the row spacing of ca. 60 cm. However, the spacing between plants was varied among locations and farmers. This study was attempted to evaluate the effect of plant density on the yield of taro grown in the paddy field[(4,5,6,12,13)].

Material and Methods

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The most common cultivar Betelnut was planted in the loam soil of the research farm of Fengshan Tropical Horticultural Experiment Station on March 6, 1979 and January 18, 1980. A randomized complete block design with four replications were used in the experiments. Each experiment unit was 3.6 m × 5 m containing 6 rows of plants with 60 cm between rows. The spacing between plants were adjusted to 20 , 30, 40, 50 and 60 cm which resulted in the density of 83,300 ; 53,300 ; 40,000 ; 33,300 and 26,600 plants/ha, respectively. Seedlings were obtained from the nursery of last year. Twenty-five tons of compost, 6 tons of chicken manure, 500 kgs of ammonium sulfate, 1,000 kgs of calcium superphosphate and 200 kgs of potassium chloride per hectare were used. All composts and chicken manures, 50% of ammonium sulfate, 80% of calcium superphosphate and 50% of potassium chloride were broadcasted as basal fertilizer during land preparation. The remainder fertilizers were applied 1, 3 and 5 months after planting. Crop was harvested on November 6, 1979 and September 11, 1980 respectively. Important horticultural characters, such as size of main corm, fresh weight of main corm, cormels, main corm + cormels, number of cormels, size of cormels, plant height and yield of main corm, cormels, main corm + cormels were recorded during harvest. All investigation data were processed by the methods of Duncans' multiple range test. Crop was managed in accordance with general cultivation of paddy field taro.

Results

As shown in Table 1, the width of main corm was increased as plant density decreased. However, no significant difference in length of main corm was observed. Table 2 showed that no difference was observed in the fresh weight of main corm per plant produced on each treatment. However, the fresh weight of cormels or main corm plus cormels was increased as plant density decreased. Number of cormels per plant was increased as plant densities descreased in the experiment of 1979 but no difference was observed in the test of 1980 (Table 3). No difference in fresh weight per cormel was observed among plant densities in the experiment of 1979 but it was increased as plant density decreased in 1980 (Table 4). Plant height was found positively correlated to the plant density (Table 5). Table 6 showed that the yield of main corm increased as the plant density increased. However, there was no difference in cormel production among plant densities. The main corm yield showed the highest in the plot with the spacing of 60 × 20 cm (83,300 plants/ha) of 36 tons/ha and 55 tons/ha and the lowest in the spacing of 60 × 60 cm (26,600 plants/ha) of 17 tons/ha and 21 tons/ha respectively in 1979 and 1980. If both main corms and cormels were used in the calculation of production, total yield was increased with increased plant density. The total yield (main corm+cormels) showed the highest in the spacing of 60 × 20 cm of 43 tons/ha and 63 tons/ha and the lowest in the spacing of 60 × 60 cm of 22 tons/ha and 26 tons/ha respectively in 1979 and 1980.

Discussions

Many factors such as fertilizer, herbicide, spacing etc. were regarded to be able to affect taro growth and yield[7,8,9,10]. Plant density might be the most important. Horti-

cultural characters of Betelnut taro, such as size (length and width) of main corm, cormels and main corm plus cormels per plant, number of cormels per plants, fresh weight per cormel, plant height and yield were strongly influenced by the plant density of taro grown in paddy fields. Some farmers thought the size of main corm per plant will be increased when density is decreased. However, no difference of fresh weight of main corm wes observed in both of the experiments conducted in 1979 and 1980. Since this variety is mainly used for the production of main corm, therefore, a higher yield is derived from a higher number of plant density plot. The main corm production per hectare for the plots with 60 × 20 cm (83,300 plants/ha) spacing was 2 times more than that of 60 × 60 cm (26,600 plants/ha) spacing plot. The most suitable plant density for the plants of Betelnut taro in paddy field in Southern Taiwan was 60 × 20 cm or 83,300 plants per hectare. This information is valuable for the farmers to cultivate taro in the paddy fields.

Acknowledgements : The author's thanks are due to Mr. T. F. Sheen, Director of the Station, Dr. Samson, C. S. Tsou, Chemist and Program Leader of Nutrition, Environment and Management, AVRDC, and Mr. C. H. Lin, Dept. head of the station for revising the manuscript.

Table 1. Effect of plant density on the length and width of main corm

Plant density (cm)	Length (cm)		Width (cm)	
	1979	1980	1979	1980
60×20	15.3 a	16.6 a	7.6 c	8.7 d
×30	17.7 a	17.5 a	8.4 abc	9.2 abc
×40	17.6 a	18.2 a	8.4 abc	9.3 ab
×50	17.6 a	18.1 a	8.6 ab	9.5 a
×60	17.8 a	17.6 a	8.8 a	9.5 a
FLSD 5%	N S	N S	0.8225	0.4404
1%	N S	N S	1.3649	0.7309

Table 2. Effect of plant density on the fresh weight of main corm, cormels and main corm + cormels per plant g/plant

Plant density (cm)	Main corm		Cormels		Main corm + cormels	
	1979	1980	1979	1980	1979	1980
60×20	427.5 a	662.9 a	84.6 d	96.8 c	512.1 b	759.7 c
×30	555.8 a	738.1 a	121.8 bcd	129.3 bc	677.6 ab	867.4 abc
×40	578.7 a	802.4 a	161.2 abc	154.3 abc	739.9 ab	956.7 abc
×50	565.9 a	793.8 a	170.4 ab	205.0 a	736.3 ab	998.8 a
×60	623.4 a	778.5 a	193.1 a	188.9 ab	816.5 a	967.4 ab
LSD 5%	N S	N S	49.7405	74.7457	238.6524	200.7188
1%	N S	N S	82.5447	124.0411	396.0458	333.0947

Table 3. Effect of plant density on the number of cormels per plant

Plant density (cm)	No. of cormels per plant	
	1979	1980
60×20	3.0 c	3.3 a
×30	4.1 abc	3.5 a
×40	4.8 ab	4.0 a
×50	4.5 abc	3.6 a
×60	5.1 a	4.1 a
LSD 5%	1.8737	N S
1%	3.1049	N S

Table 4. Effect of plant density on the average fresh weight of cormel

Plant density (cm)	Fresh weight/cormel (g)	
	1979	1980
60×20	29.6 a	29.6 b
×30	29.3 a	36.8 ab
×40	33.4 a	39.2 ab
×50	38.9 a	54.6 a
×60	38.1 a	47.8 ab
LSD 5%	N S	21.5739
1%	N S	35.8022

Table 5. Effect of plant density on the plant height of lowland taro

Plant density (cm)	Plant height (cm)	
	1979	1980
60×20	142.3 a	143.3 a
×30	136.9 ab	138.6 ab
×40	135.4 b	138.3 abc
×50	132.8 b	132.8 d
×60	131.1 b	131.0 d
LSD 5%	6.8849	5.2044
1%	11.4255	8.6368

Table 6. Effect of plant density on the yield of lowland taro

(ton/ha)

Plant density (cm)	Main corm		Cormels		Main corm + cormels	
	1979	1980	1979	1980	1979	1980
60×20	35.6 a	55.2 a	7.1 a	8.1 a	42.7 a	63.3 a
×30	29.6 ab	39.3 b	6.5 a	6.9 a	36.1 ab	46.2 b
×40	23.2 bc	32.1 bc	6.4 a	6.2 a	29.6 bc	38.3 bc
×50	18.9 c	26.5 cd	5.7 a	6.9 a	24.6 c	33.4 cd
×60	16.6 c	20.7 d	5.2 a	5.1 a	21.8 c	25.8 d
LSD 5%	9.6202	7.6571	N S	N S	11.0817	9.0830
1%	15.9648	12.7071	N S	N S	18.3902	15.0733

Literature Cited

1. Chen, P. C. 1978. Taro cultivation. Growing of Stem vegetables. Harvest Farm Magazine. Book Division HV #781. p. 166–173. (In Chinese).
2. Dept. of Agriculture & Forestry. Provincial Government of Taiwan. 1981. Taiwan Agriculture Year-Book. p. 195. (In Chinese).
3. Herklots, G. A. C. 1972. Taro. Vegetables in South-East Asia. London George Allen & Unwin Ltd. p. 404–412.
4. Liou, T. D. 1979. Lowland taro culture. Harvest Farm Magazine 29(22) : 28–29. (In Chinese).
5. Liou, T. D. 1979. Pests control of lowland taro. Harvest Farm Magazine 29(23) : 38–39. (In Chinese).
6. Liou, T. D. 1980. Taro cultivation. Farmers Extension Bulletin. Dept. of Agriculture & Forestry. Provincial Government of Taiwan. 23pp. (In Chinese).
7. Pena, R. S. de la. 1975. Nitrofen for taro weed control. Cooperative extension service, University of Hawaii, Circular 488. 13pp.
8. Pena, R. S. de la and D. L. Plucknett. 1967. The response of taro (*Colocasia esculenta* (L.) Schott) to N, P and K fertilizer under upland lowland conditions in Hawaii. Proceedings of International Symposium on Tropical Root Crops. p. II–70–85.
9. Pena, R. S. de la and D. L. Plucknett. 1972. Effects of nitrogen fertilization on the growth, composition and yield of upland and lowland taro (*Colocasia esculenta*). Exp. Agric. 8 : 187–194.
10. Pena, R. S. de la, D. L. Plucknett and G. H. Shibao. 1971. Application of herbicides through irrigation water for weed control in lowland taro (*Colocasia esculenta*). Proceedings of the Asian Pacific Weed Science Society No. 3, Kuala Lumpur. p. 332–334.
11. Plowman, T. 1969. Folk uses of new world aroids. Economic Botany 23(2) : 97–122.
12. Plucknett, D. L., R. S. de la Pena and F. Obrero. 1970. Taro (*Colocasia esculenta*). Field Crop Abstracts 23(4) : 413–426.
13. Plucknett, D. L. and R. S. de la Pena. 1971. Taro production in Hawaii. Hawaii Agricultural Experiment Station. Journal Series No. 1348, 6pp.
14. Yu, C. H. 1980. Taro cultivation. Vegetables Growing. Revised 3rd Edition. Harvest Farm Magazine. Book Division HV #752-3 p. 71–77. (In Chinese).

15. Yu, C. H., W. Y. Chen, T. C. Tseng and T. Chen. 1952. A survey on taro cultivation in central and southern Taiwan. Published by Fengshan Tropical Horticultural Experiment Station. TARI. (In Chinese).

水芋種植密度對產量之影響 [1]

劉 政 道 [2]

摘 要

檳榔心芋於1979及1980年分別種植於行距 60公分，株距 20，30，40，50及60公分即每公頃種植83,300株；53,300株；40,000株；33,300株及26,600株等五種不同種植密度之水田，試驗採用逢機完全區集設計四重複，每小區種植 6 行，行長 5 公尺，小區面積爲18平方公尺。兩年之試驗結果顯示植株之高度與產量均隨種植密度之增加而顯著增加，然而單株母芋或子芋之大小、鮮重或數目均與種植密度呈負相關之趨勢，母芋之產量或母芋加子芋之總產量均以60公分×20公分即每公頃種植83,300株者爲最高，而以60公分×60公分即每公頃種植26,600株者爲最低，此資料顯示在臺灣南部地區檳榔心芋水田栽培之最適種植距離應爲60公分×20公分即每公頃種植83,300株，此可提供農民種植水田芋頭之依據及學術研究之參考。

1. 臺灣省農業試驗所 研究報告第1143號。
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中華農業研究（Jour. Agric. Res. China）33(1)：044～052（1984）

水田及水稻根部微生物固氮之研究[1]

林　　錫　　錦[2]

摘要：水稻根部微生物固氮力弱，供試８個品種間固氮能力於每次採樣時品種間固氮活性之高低無一固定的傾向，水稻抽穗前之固氮活性較其他生長期高，70年第一期稻作之固氮活性較69年第二期稻作低。施用氮肥（120 kg/ha）抑制根部微生物之固氮活性。由水稻根部分離到一種普遍存在於根上之革蘭氏陰性短桿固氮菌。

水田田土固氮力微弱，田水無固氮力。種植水稻之水田田土之固氮力比不種植水稻者高些，尤其以水稻抽穗前最顯著，在水稻生育期中，根圈土之固氮活性比底土者高。由水田田土分離到固氮藍綠藻 *Anabaena* sp. 及 *Nostoc* sp.，以及一種廣泛存在於水田田土之革蘭氏陰性短桿固氮細菌。接種十種不同的固氮藍綠藻對盆栽水稻生長效應之研究，發現固氮藍綠藻 HN 801 品系對水稻之株高、穗數及穀產量之肥效約等於或超過施用半量化學氮肥（N 60kg/ha）者。

現代之水稻栽培方法中已習慣化學氮肥的大量使用，但水稻生長期中仍自土壤中吸取了過半量其生育期中所需的氮素。據菲律賓國際稻米研究中心試驗結果，在同一土地上連續種植23期稻作，未施氮肥，稻穀產量並未減產[12]，顯然由於微生物固氮而維持地力不衰。這些水稻生長期中所吸收的氮素來自土壤中腐植質，前作殘留藁根分解時與固氮細菌共生，以及水稻根部與水田中林林總總的固氮微生物，如固氮細菌、藍綠藻、滿江紅等所累積之氮肥[1,2,3,11,13]。由於化學氮肥製造過程須消耗大量的能源殊為不經濟，既然水稻及水田本身的固氮微生物可提供多量氮素，因此對水稻根部微生物的固氮、水田固氮微生物的瞭解、保護及强化其固氮功能實為今日水稻栽培必須重視的一大課題。本省水稻栽培亦偏用化學氮肥，而且大量農藥的使用很可能造或對固氮微生物生態之破壞。有關水稻根部固氮國外文獻業已證實。[4,5,6,7,8,9,10,14]，本省對於水稻根部固氮及水田微生物固氮尚無資料可供參考，本實驗乃著手探討：（１）水稻根部微生物固氮之情形及水稻不同品種間固氮能力之差異，提供育種與農民選種及施肥之參考，並希望從中分離出固氮力强之細菌。（２）對水田一般固氮微生物之固氮力加以廣泛瞭解，藉以篩選其中具有高固氮力之微生物加以培養、保存，提供日後進一步試驗之材料。

材料與方法

一、水稻根部微生物固氮之試驗

（一）田間試驗：於農試所25號圃場進行

1.供試品種有高雄１號、臺中秈３號、臺南５號、臺東27號、臺東28號、新竹56號、臺農67號及高雄139號。

1.臺灣省農業試驗研究報告第1144號，本文第一部份承國科會 NSC-70-0409-B055-04 補助，第二部份承農發會71農建一４、１一產38補助，謹誌謝忱。

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2. 試驗方法：

（1）種植期：69年第二期作及70年第一期作。

（2）施肥法：分別施用 $N—P_2O_5—K_2O=120—60—60$ kg/ha 及不施用氮$N—P_2O_5—K_2O=0—60—60$ kg/ha ，氮肥用硫酸銨，其中 45%當基肥，30% 於插秧後一個月施用，25%於插秧後二個月施用，磷肥用過磷酸鈣，悉數基肥。鉀肥用氯化鉀，分兩次使用，其中 60% 基肥，40%於插秧後一個月施用。

（3）田間設計：施 N 肥與不施 N 肥兩種處理，每品種四重複，完全逢機法行之。每小區面積 9 平方公尺。

（二）室內試驗：

1. 從分蘖初期至抽穗期，分適當時間至田間採取根部回實驗室，沖洗泥土後置於 680 ml 血清瓶中，套以塑膠塞，抽出 68 ml 空氣，並打入68 ml 之乙炔氣體，於26 °C下孵育一天後取出 1 ml 氣體，打入氣體色層分析儀中，測定乙稀生成量，並換算成固氮酵素之固氮活性。

2. 固氮細菌之分離：以無氮之培養基，分離純化根部之固氮細菌。

二、水田微生物固氮之試驗

（一）田間試驗於本所20號圃場進行。

（二）試驗設計：

1. 分為四種處理，每種處理面積 6.5 平方公尺，逢機區集設計，各處理為

（1）不施氮肥，種水稻。

（2）不施氮肥，不種水稻，施用丁基拉草殺草劑 30 kg/ha。

（3）標準施肥區(N 120 kg/ha，45%基肥，30% 追肥，25% 穗肥)，種水稻。

（4）施半量氮肥　N 60 kg/ha，悉數基肥），種水稻。

2. 氮肥施用硫酸銨，各區均施磷鉀肥，其用量為 P_2O_5 80 kg/ha，悉數基肥，施用過磷酸鈣。鉀肥用量為 K_2O 60 kg/ha，其中60% 基肥、40% 追肥，施用氯化鉀。每小區水稻種植品種共有臺農67號及臺中秈 3 號，以標準插秧法插植。

（三）水田田土及田水固氮活性之測定：於水稻分蘖初期、中期及盛期，及孕穗期至水稻田採取根圈土及根圈以外的底土各25克，根圈土及底土每次取樣各取 3 重複，以乙炔還原法測定其固氮力。以瞭解水田固氮微生物之固氮活性情形。測定時以50 ml 三角瓶內裝 25 cm 高之田土或田水，然後塞以橡膠瓶塞，抽出 4.5 ml 之空氣再打入 4.5 ml 之乙炔，置於室內照明架上一天（其中光照 18 小時），然後抽出 1 ml 之混合氣體打入氣層分析儀中測定乙稀生成量，再換算為固氮量。

（四）固氮微生物之分離及篩選：攜回已知固氮力之樣品中，先於去氮培養液（視固氮微生物種類而決定所用培養液）中淘汰非固氮之其他微生物，再將所留固氮微生物分離出來，純粹培養後再以乙炔還原法測定其固氮力之强弱。

（五）接種固氮藍綠藻對水稻生長效應之試驗：將10種不同之固氮藍綠藻（由本實驗室分離得到，及由中央研究院黃檀溪博士所分離提供）接種於盆栽水稻，探討其對水稻株高、穗數及穀產量之影響。水稻品種用臺農67號，試驗採完全逢機盆栽設計，13處理：即接種10種不同之固氮藍綠藻，施用全量氮肥（N 120kg/ha），施用半量氮肥（N 60 kg/ha）及不施氮肥亦不接種固氮藍綠藻。

結果與討論

一、水稻根部微生物固氮試驗

水稻田間試驗，從分蘖期至抽穗期分數次採樣做根部微生物固氮之測定，並且比較其品種間固氮

力之差異，其結果如表 1 及表 2 。水稻根部微生物固氮力弱，可能因爲在水田下缺乏氧氣，好氣性的根部固氮菌難於生存，另外嫌氣性的根部固氮菌亦少之故。每次採樣時品種間固氮率之高低均無一定傾向。69年二期作（表 1 ）第一次採樣之固氮活性有的品種比其他時期高，有點奇怪。第二次採樣（

表1. 69年度第二期作稻根之固定氮率 (1)

品種	n mole N_2 fixed/g fresh root/day 採樣期											
	9/4		9/12		9/18		9/27		10/3		10/9	
	+N (2)	−N	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N
高雄 1 號	31	21	0	3	4	10	4	11	4	10	9	15
臺中秈 3 號	14	18	1	4	10	27	15	9	7	10	15	24
臺南 5 號	13	37	0	6	4	18	10	18	6	13	18	10
臺東27號	22	27	0	4	3	21	6	33	3	6	7	19
臺東28號	19	22	1	4	6	28	12	13	7	18	15	39
新竹56號	4	24	1	9	6	24	34	18	13	21	28	36
臺農67號	4	31	0	7	10	30	40	16	13	18	28	33
高雄選139號	9	21	0	6	4	22	18	18	10	27	15	13
L. S. D.	15.3		—		6.67		13.91		7.62		10.9	

（1）插秧日期：7月30日　　（2）每公頃施氮肥120公斤。

9 月12日）之固氮活性比第一次急速下降，其原因可能爲第一次採樣後鄰近水稻田施肥，下雨後中間的田埂溢水流入本試驗田，氮肥抑制固氮菌之固氮活性。70年一期稻作各品種間之固氮活性除了抽穗前（5 月13日）的採樣外，其餘各採樣期其固氮活性均比69年第二期稻作低。表 1 中施用氮肥區各品種間之固氮活性比不施氮肥低，此可能因爲氮肥減低微生物固氮酵素之活性。可是每次採樣時施氮與否之區，因重複間之差異極大，結果不顯著，但是品種間有顯著的差異。

70年第一期作施氮肥區與不施氮肥區其固氮活性無顯著之差異（表 2 ），其原因可能因施肥後下

表2. 70年度第一期作稻根之固氮率 (1)

品種	n mole N_2 fixed/g fresh root/day 採樣期							
	4/13		4/23		5/13		5/26	
	+N (2)	−N	+N	−N	+N	−N	+N	−N
高雄 1 號	0.7	0.1	0.6	0.6	20.5	48.9	0.8	1.6
臺中秈 3 號	0	0.1	0.1	1.6	34.2	42.8	2.0	1.7
臺南 5 號	0.3	0	0.5	1.5	33.1	27.2	1.4	1.1
臺東27號	0.1	0.2	0.7	0.8	25.9	63.8	2.0	1.8
臺東28號	0.1	0.2	0.7	1.7	49.5	52.0	1.6	1.9
新竹56號	0.2	0.2	0.6	1.6	33.8	29.8	1.7	1.0
臺農67號	0.4	0.2	1.0	3.2	25.7	29.7	2.2	0.8
高雄139號	0.2	0.1	0.4	1.6	31.1	25.3	2.1	1.3
L. S. D.	—		—		14.27		—	

（1）2月23日插秧。　　（2）氮肥每公頃施用 120公斤。

雨，田水溢流入無氮肥區，結果稻株生長濃綠。69年二期作及70年一期作各採樣期之固氮活性均以水稻抽穗前（分別爲9月27日到10月9日，以及5月13日）（表1及表2）固氮活性最高，其餘採樣期間其固氮活性均甚低。由69年二期作及70年一期作觀察水稻之生育情形，不施氮肥區各品種間發育均呈萎黃缺乏氮肥之現象，株高及結穗亦較施氮肥區低。

綜觀田間試驗之結果，水稻根部微生物固氮率甚弱。蓋水稻爲禾本科鬚根作物，其根圈之固氮細菌爲非共生固氮菌，在水田嫌氣情況下，主要之固氮菌爲嫌氣性細菌或半嫌氣性細菌及少數好氣性細菌。這些共生固氮細菌不易在稻鬚根上形成大族羣的固氮細菌，因而固氮率弱。不像豆科作物能够與根瘤菌共生產生根瘤進行多量的固氮。然而菲律賓國際稻米研究中心試驗結果，謂在同一土地上連續種植23期稻作，未施氮肥稻穀產量並未減產（10）。顯然的維持地力不衰的原因可能包括（1）稻桿埋入田中，纖維分解菌與固氮細菌共生，累積一些氮肥於土壤中，例如本人1976年在華盛頓州立大學與Dr. Peter Wong 從事固氮研究工作時，在當地的麥田採取分解中的麥桿，發現在麥桿分解期間有很强的固氮作用，一直到麥桿完全分解後固氮力才消失，在土壤中累積一些氮肥。在實驗室研究的結果發現分解麥桿菌與固氮細菌營共生現象。據中央研究院植物研究所黃檀溪博士謂稻草分解時也有不小的固氮作用。（2）本實驗室70年至71年做水田微生物固氮之研究時，觀察到稻田根圈土及底土亦有一些固氮活性，其中根圈土之固氮活性比底土高並從水稻根部分離到一種普遍存在於根上之革蘭氏陰性短桿固氮細菌（圖1）。其固氮活性隨着水稻長大而增加。（3）各種固氮藍綠藻及固氮細菌在稻田中亦多少會增加一些氮肥供給水稻。

二、水田微生物固氮之試驗

本試驗之目標首先著重於水稻生育過程中田土固氮力變化之探討與固氮微生物之分離。

（一）水田田土及田水固氮能力之分析：

1. 各處理之水田田水均無固氮活性。水田田土固氮力微弱。

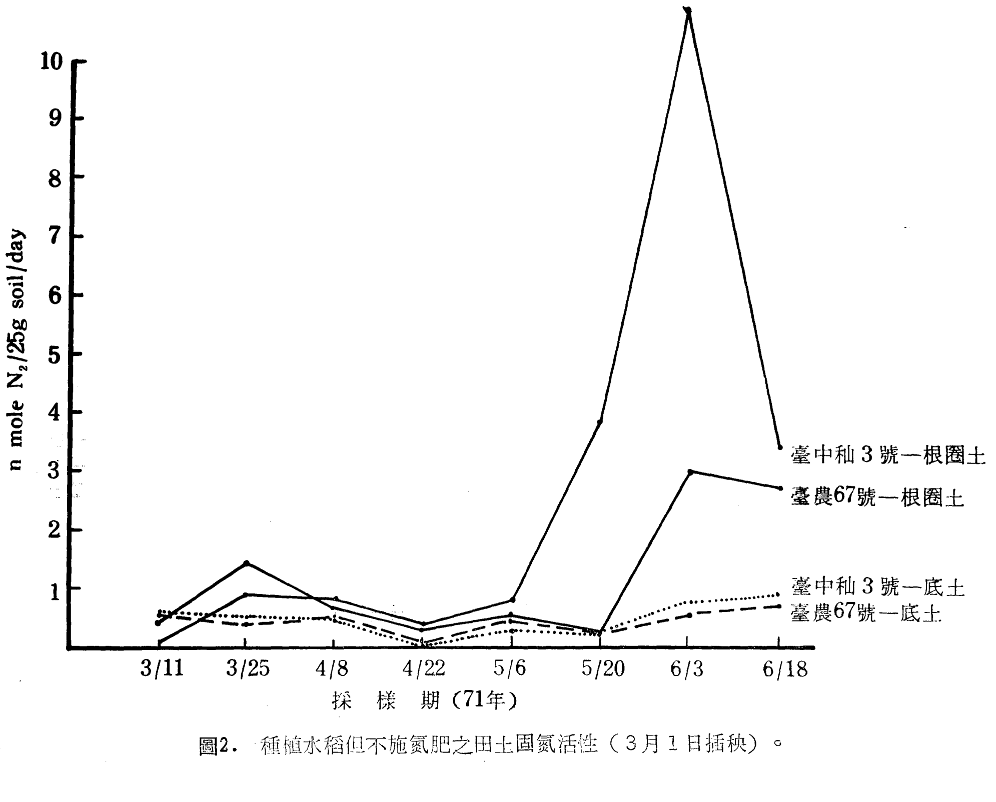

圖2. 種植水稻但不施氮肥之田土固氮活性（3月1日插秧）。

2.種植水稻區（圖2、圖4及圖5之臺中秈3號）之根圈土，其固氮活性以抽穗前的採樣分析（分別為5月20日及6月3日）差異較顯著，其他時期之根圈土固氮活性無差異。此可能因為水稻生長到抽穗前，植株及根部的生長為最大的時期，根部分泌一些養分供給固氮細菌生長繁殖，土壤中非共生固氮細菌的族羣增大，因而其固氮活性增高。底土之固氮活性則種植水稻區（圖2、圖4及圖5）與不種植水稻區（圖3）無差異。

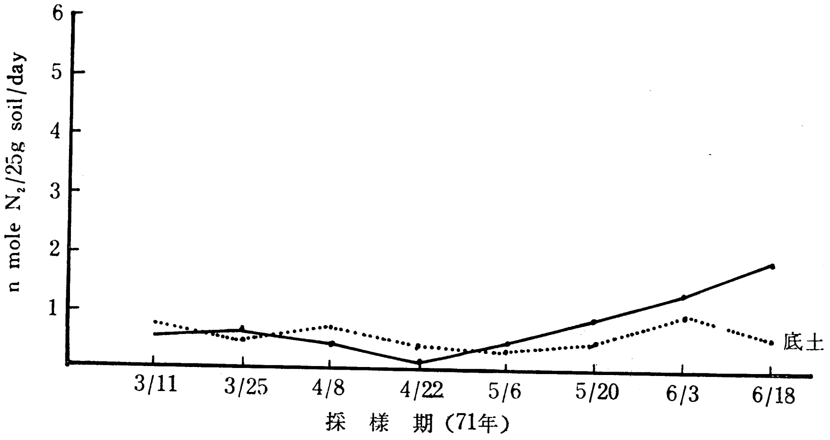

圖3. 不種水稻與不施氮肥但使用殺草劑之田土固氮活性。

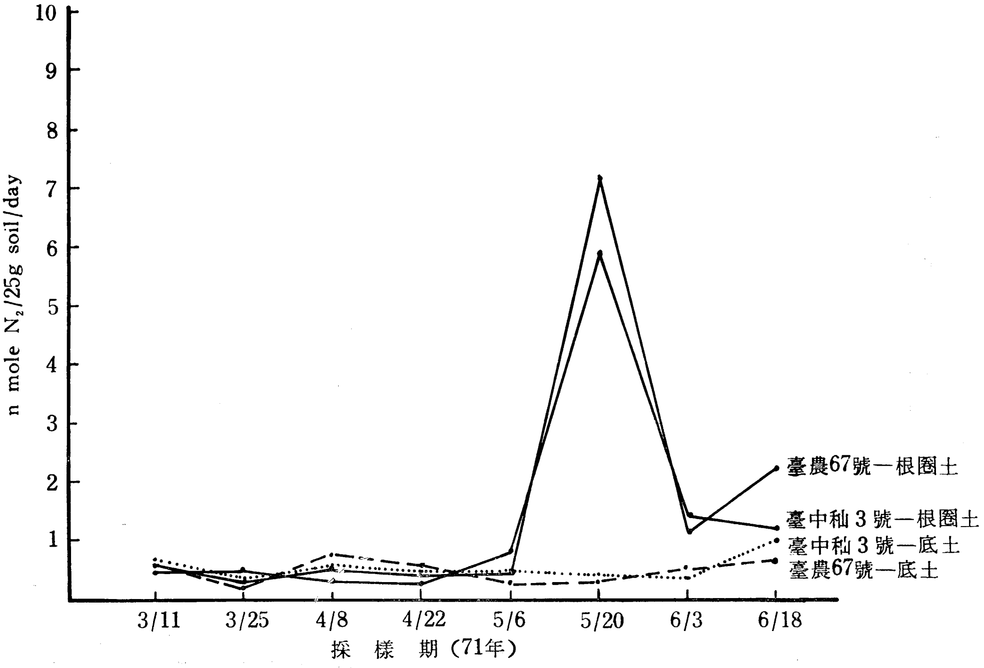

圖4. 種植水稻與施用全量氮肥（N 120 kg/ha）之田土固氮活性（3月1日插秧）。

3.臺中秈3號之根圈土固氮活性在抽穗前採樣之不施氮肥區（圖2之6月3日）比施全量氮肥區（圖4之5月20日）及半量氮肥區（圖5之6月3日）較高。同時期採樣之臺農67號的根圈土固氮活性則以施全量氮肥區（圖4）比不施氮肥區（圖2）或施半量氮肥

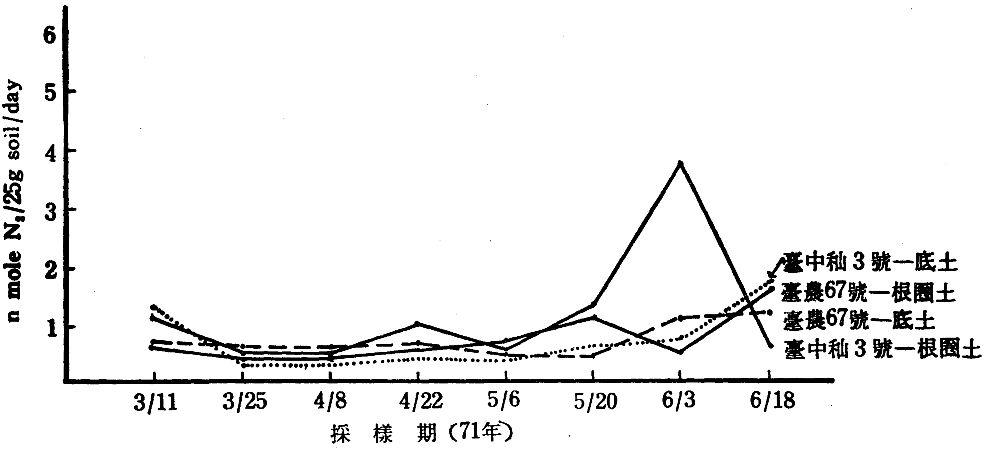

圖5. 種植水稻以及施用半量氮肥(N 60kg/ha)之田土固氮活性(3月1日插秧)。

區(圖5)較高。施用全量氮肥區的臺中秈3號及臺農67號之根圈土固氮活性最高期為5月20日(圖4)比施半量氮肥區或不施氮肥區(圖5及圖2之6月3日)之根圈土最高固氮活性早兩星期,此可能為施用半量氮肥區或不施氮肥區之水稻植株生長不好,抽穗期比施全量氮肥者晚兩星期,而抽穗前為根部分泌能供給固氮細菌養分最多的時候。

4.綜觀水田田土固氮力極微弱,底土之固氮活性在四種不同之處理下,其固氮活性均不到 1 nmole N_2/25g soil/day(等於 N 2.24 kg/ha/一期作)(圖2、圖3、圖4及圖5)。在種植水稻區之根圈土固氮活性抽穗前為最高,可是也只有 3.5~10.2 nmole N_2/25g soil/day(等於 N 7.84~22.85 kg/ha /一期作)。在水田嫌氣情況下,主要之固氮菌為嫌氣性細菌或半嫌氣性細菌及少數好氣性細菌。這些非共生固氮細菌不易形成大族羣的固氮細菌,因而固氮率極弱。而且水稻為鬚根作物不像豆科作物能够與根瘤菌共生產生根瘤進行多量的固氮,其結果與原因已如上面第一部分討論中所述。

(二)固氮藍綠藻之分離:一般土壤中固氮藻以絲狀藍綠藻如 Anabaena sp. 及 Nostoc sp. 等居多。這些絲狀藻多會產生粘液,與其他雜藻混生。因此分離之工作頗為困難。此點以往之研究曾予以討論過。本研究室在進行田土固氮藍綠藻分離時發展出一套方法可有效分離且純化出固氮藻來。其方法如下:欲分離之樣品(包括田土、田水或混生藻團)取少量於去氮之 T 培養液(KH_2PO_4, 0.6g;$MgSO_4$, 0.5g;EDTA-Fe,1ml;微量元素,0.1 ml)內於 700 lux 光照下培養,俟藻類長至肉眼可見時,再以乙炔還原法測定其有無固氮力。然後以顯微鏡鏡檢。一般此時長出多為固氮藻類,但仍有許多雜藻混生。進一步純化方法是將混有雜藻之藻團以均質機打碎 0.5—1分鐘,此時藻團完全打散,固氮藻亦被打成許多小截,稍微稀釋後取一滴滴於水瓊脂平板上,在50倍之解剖顯微鏡下以微細玻棒挑出單截固氮藻,移植於 T 培養液或斜面上,2—3星期即可長成菌落,長出之菌落再以顯微鏡鏡檢,可確定此菌落是否雜有其他藻類,以此方法進行固氮藍綠藻之分離獲得相當良好之結果。由本研究分離到 Anabaena sp.(圖6)及 Nostoc sp.(圖7)之固氮藍綠藻。

(三)接種固氮藍綠藻對水稻生長效應之試驗:

接種10種不同固氮藍綠藻對盆栽水稻生長效應之研究,發現固氮藍綠藻 HN 801 之品系對水稻之株高、穗數及穀產量之肥效等於施用半量化學氮肥(N 60kg/ha)者(圖9)其餘9種固氮藍綠藻對水稻之肥效不顯著。有關 HN 801 固氮藍綠藻之生理,生化特性及對水稻生長有

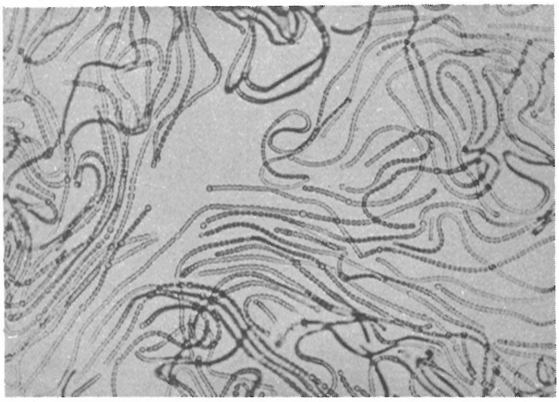

圖6. 由水稻根上分離到的一種廣泛存在於稻根上的革蘭氏陰性短桿固氮細菌。

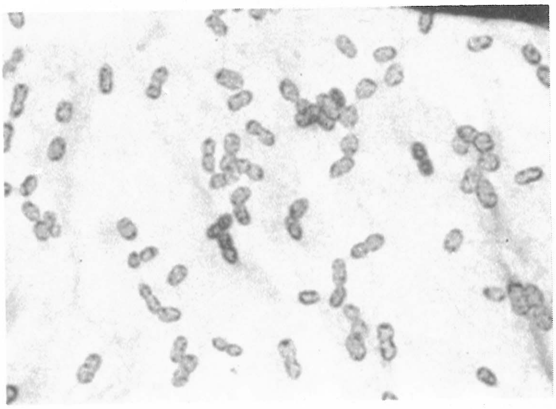

圖7. 分離得到廣泛存在於水田田土的一種固氮藍綠藻 Anabaean sp, 正加以繁殖接種到盆栽水稻對水稻生長效應之研究中。

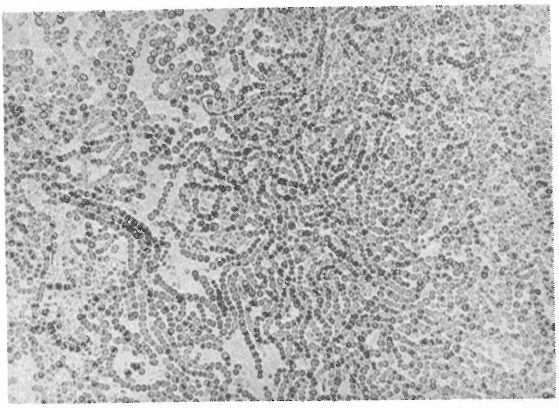

圖8. 分離得到廣泛存在於水田田土的一種固氮藍綠藻 Nostoc sp., 正加以繁殖接種到盆栽水稻對水稻生長效應之研究中。

圖9. 接種固氮藍綠藻 HN 801 對水稻生長之效應。盆栽由左至右爲：全 N=施用化學氮肥 N 120 kg/ha ；半 N=施用化學氮肥 N60 kg/ha；—N=不施肥亦不接種固氮藍綠藻；HN 801 及 HN 801=接種固氮藍綠藻 HN 801 品系，不施肥料。

利之原因正進一步探討中。

(四) 好氣性固氮細菌之分離：分離方法與固氮藻者相似，但使用之培養液係在 T 培養液內加入 10 g/l 之 Glucose（簡稱 Tg 培養液），且樣品加入培養液即置於黑暗中培養，以除去藻類生長。5－6 天後以乙炔還原法測其固氮活性，對有明顯固氮力之樣品再以稀釋平板法在 Tg 平板上，俟長出菌落後在解剖顯微鏡下挑出各型菌落，培養於 Tg 半固體培養基內。3－4 天，再以乙炔還原法測定挑出各菌，是否有固氮力。由本研究分離到一種廣泛存在於根圈土的革蘭氏陰性短桿固氮細菌。

參考文獻

1. 川口桂三郎，1980，水田之氮固定作用，土壤肥料通訊324：1689—704（洪崑煌譯）
2. 黃啓穎1977，藍綠藻之固氮作用，科學農業25（9—10）280—286
3. 黃檀溪1982，水田固氮藍綠藻之生態探討，科學發展月刊10(7)，619—631。
4. Balandreau, J., Rinaudo, G., Fares-Hamad I. and Dommergues, Y. 1975. N_2-fixatior in the rhizosphere of rice plants. In Nitrogen-fixation by free-living microorganisms (W. D. P. Stewrat. Ed.) pp. 57-70. Cambridge University press.
5. Brill, W. J. 1977. Biological N_2-fixation. Scient. Am. 236, 68-81.
6. Dommergues, Y., Balandreau, J., Rinaudo, G. and Weinhard, P. 1973. Non-symbiotic N_2-fixation in the rhizosphere of rice, maize and different grasses. Soil Biol. and Biochem. 5, 83-89.
7. Gilmour, J. T., C. M. Gilmour and T. H. Johnston. 1978. Nitrogenase activity of rice plant root systems. Soil Biol. Biochem. Vol. 10 pp. 261-264.
8. Hirota, Y., T. Fujii, Y. Sano., and S. Iyama. 1978. N_2-fixation in the rhizosphere of rice. Nature 276 : 416-417.
9. Ito, O., D. C. Cabrera, and I. Watanabe. 1980. Fixation of dinitrogen-15 associated with rice plants. Appl. Environ. Microbiol. 39(3) : 350-355.
10. Lee, K. K., T. Castro, and T. Yoshida. 1977. N_2-fixation throughout growth and varietal differences in N_2-fixation by the rhizosphere of rice planted in pots. Plant and Soil 48 : 613-619.
11. Watanabe, A. 1962. Effect of Nitrogen-fixing blue-green algae : Tolypothrix tennis on the nitrogenous fertility of paddy soil and on the crop yield of rice plant. Jour. Gen. Appl. Microbiol. 8(2) : 85-91.
12. Watanabe, I., K. K. Lee, B. V. Alimagno, M. Sato, D. C. del Rosario, and M. R. de Guzman. 1977. Biological N_2-fixation in paddy field by in situ acetylene-reduction assays. IRRI. Research papers series No. 3.
13. Yoshida, T. 1975. Biological source of nitrogen in natural ecosystem and crop production. FAO Soil Bull. 27 : 165-174.
14. Yoshida, T. and R. R. Ancajas. 1971. N_2-fixation by bacteria in the root zone of rice. Soil Sci. Soc. Am. Proc. 35 : 156-157. .

Studies of the Microbial Nitrogen-fixation in Rice Roots and Rice Paddies[1]

Chyi-Jiin Lin[2]

Summary

The N_2-fixation rate of the excised rice root was low. The difference of N_2-fixation rate among the eight rice varieties varied in every samplings during rice growth stages, and none have shown a definite trend of higher N_2-fixation rate in every samplings. The nitrogenase activity was highest in the booting stage. The N_2-fixation rate during the first rice crop-season of 1981 was lower than that of the second rice crop-season of 1980. The microbial nitrogenase activity of the rice root was inhibited with the application of 120 kg/ha of N fertilizer. A gram negative rod bacterium which is widely existed on the rice root and responsible for the nitrogen-fixation in the rice root was isolated.

Nitrogen-fixation rate in the rice paddies was very low. No nitrogenase activity was observed in the paddy waters. The nitrogenase activity of the paddy fields grown with rice was higher than that of the paddy fields without grown with rice, it was significantly different in the booting stage. In general, nitrogenase activity in the rhizosphere soil was higher than that of the subsurface soil. Nitrogen-fixing blue green algae such as *Anabaena* sp. and *Nostoc* sp. were isolated from the rice paddies, and a gram negative rod nitrogen-fixing bacterium which is widely distributed in the rhisosphere soil was isolated. Greenhouse experiment was conducted to investigate the effect of ten strains of N_2-fixing blue green algae on the growth response of rice plant. Inoculation of N_2-fixing blue green algae HN 801 was found to give grain yield equal to/or higher than that of the yield of applying 60kg/ha of chemical N fertilizer.

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1. Contribution No. 1144 from the Taiwan Agricultural Research Institute (TARI). The first part and the second part of this research were supported by the National Science Council (NSC-70-0409-B055-04) and the Council for Agricultural Planning and Development (71 農建 4.1—產 38), respectively, Taiwan, ROC.

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中華農業研究 (Jour. Agric. Res. China) 33(1) : 053～058 (1984)

細花野牽牛簇葉病之光學及電子顯微鏡觀察[1]

楊一郎[2] 陳脉紀 曾德賜[3]

摘要：細花野牽牛簇葉病病株及感染該病之長春草病株之葉脈組織經超薄切片與電子顯微鏡觀察結果，在其病組織之篩管細胞內發現多形性擬菌質體 (Mycoplasma-like organism) 之存在，擬菌質體直徑約爲 60～740 nm，在長春草病組織篩管細胞內較爲充滿擬菌質體。另以該長春草病組織徒手切片之 Dienes' stain 染色與光學顯微鏡觀察結果，在其篩管細胞內顯現專一性藍色反應，此項反應表示該篩管細胞中擬菌質體之存在。另在供試健全植株者則無此項反應亦無擬菌質體之存在。依此項擬菌質體之形態存在位置及對 Dienes' stain 之特殊反應與其他已知簇葉病所發現之擬菌質體相類似，該擬菌質體可能爲新記載之細花野牽牛簇葉病之病原。

細花野牽牛簇葉病 (*Ipomoea obscura* witches' broom) 係第一作者於 1977 年在臺南發現之新病害 (2,5,7)。本病在國外尙無報告，乃依其花器葉化 (phyllody) 及細葉叢生病徵稱爲細花野牽牛簇葉病，本病普遍發生於本省中南部，爲一種簇葉萎黃型病害 (7)。本病經證實可經菟絲 (*Cuscuta chinensis* Lam.) 媒介 (Cuscuta vector) 傳染而使長春草 (*Vinca rosea* L.) 罹病並顯現葉化花 (phyllody) 及極端之小葉叢生及黃化枯死之嚴重型叢生萎黃病徵 (Extremely bushy witches' broom symptoms) (5,7)。本病與本省田間發現之甘藷簇葉病 (Sweet potato witches' broom)、落花生簇葉病 (Peanut witches' broom)、絲瓜簇葉病 (Loofah witches' broom) 及國外報告之 (Aster yellows) 等叢生型萎黃病相異，爲一種在國外尙無記錄之新植物菌質病害 (1,2,3,4,5,6,14,16,17)。本文將本病病原之電子顯微鏡與光學顯微鏡觀察結果提出報告，以供植物叢生型萎黃病害研究之參考。

材料與方法

一、電子顯微鏡觀察 (Electron microscopic observations)：以細花野牽牛簇葉病病株及感染該病之長春草典型病株葉片，沿葉脈切成 5×1 mm 大小之小片，在低溫 4°C 下固定於以0.1M磷酸緩衝液 (pH 7.0) 稀釋之4 %戊二醛 (Glutaraldehyde) 及四氧化鋨 (Osmium tetroxide) 各 2 小時，以酒精系列 (Alcohol series) 脫水後包埋於 (Epon 812) 樹脂混合液內 (15)。超薄切片以 Leitz's Fernāndez-Morān type 超薄切片機進行，並以醋酸鈾 (Uranyl acetate) 及酒石酸鉛 (Lead tartrate) 染色，最後以 JEM-7 型電子顯微鏡，於 80 KV下觀察之 (9,11)。健全葉片亦以同一方法進行包埋及觀察，藉以做爲對照。

二、光學顯微鏡觀察 (Light microscopic observations)：依 Deeley *et al.* 等之方法 (6,8,12)，以

1.臺灣省農業試驗所　研究報告第 1145 號。本文爲行政院國科會補助計畫 (NSC-71-0409-B055-02) 之部分報告。
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菟絲媒介感染細花野牽牛簇葉病之典型叢生長春草病株分蘖葉脈及莖組織之徒手切片，經 0.2 % Dienes' stain 染色，另以健全長春草植株組織切片對照，經以光學顯微鏡觀察判別其病組織篩管細胞之 MLO 存在情形，並以此方法比較及證實其電子顯微鏡觀察之結果。

結　果

一、電子顯微鏡觀察：

在細花野牽牛簇葉病病株葉脈之超薄切片，發現在篩管細胞內有多變型擬菌質體 (Pleomorphic mycoplasma-like bodies) 存在，在薄壁組織或葉肉組織細胞中則未發現。健株葉脈切片亦無擬菌質體的存在。擬菌質體在篩管細胞之分佈情形不一致，菌體零散分佈，其大小型狀亦頗不一致，有小球型、圓型、橢圓形及曲管形等，菌體數量較為稀少而不充滿（圖 1.2）。在長春草病株葉脈切片之篩管細胞內，即有充滿之多形性擬菌質體，菌體在該細胞內極多量聚集充滿而呈大圓型、橢圓型及小球型等，在中型及大型菌體內可觀察到網狀絲 (Net-like strands)（圖 3）。上述菌體大小頗不一致，其直徑約為 60—740 nm，在長春草病株組織之篩管細胞之菌體分佈較多且濃度較高。此項結果與其他已報告之植物萎黃病所發現之擬菌質體（Mycoplasma-like organisms）(MLO) 相類似[9,10,11,13,14,16,17]。

二、光學顯微鏡觀察：

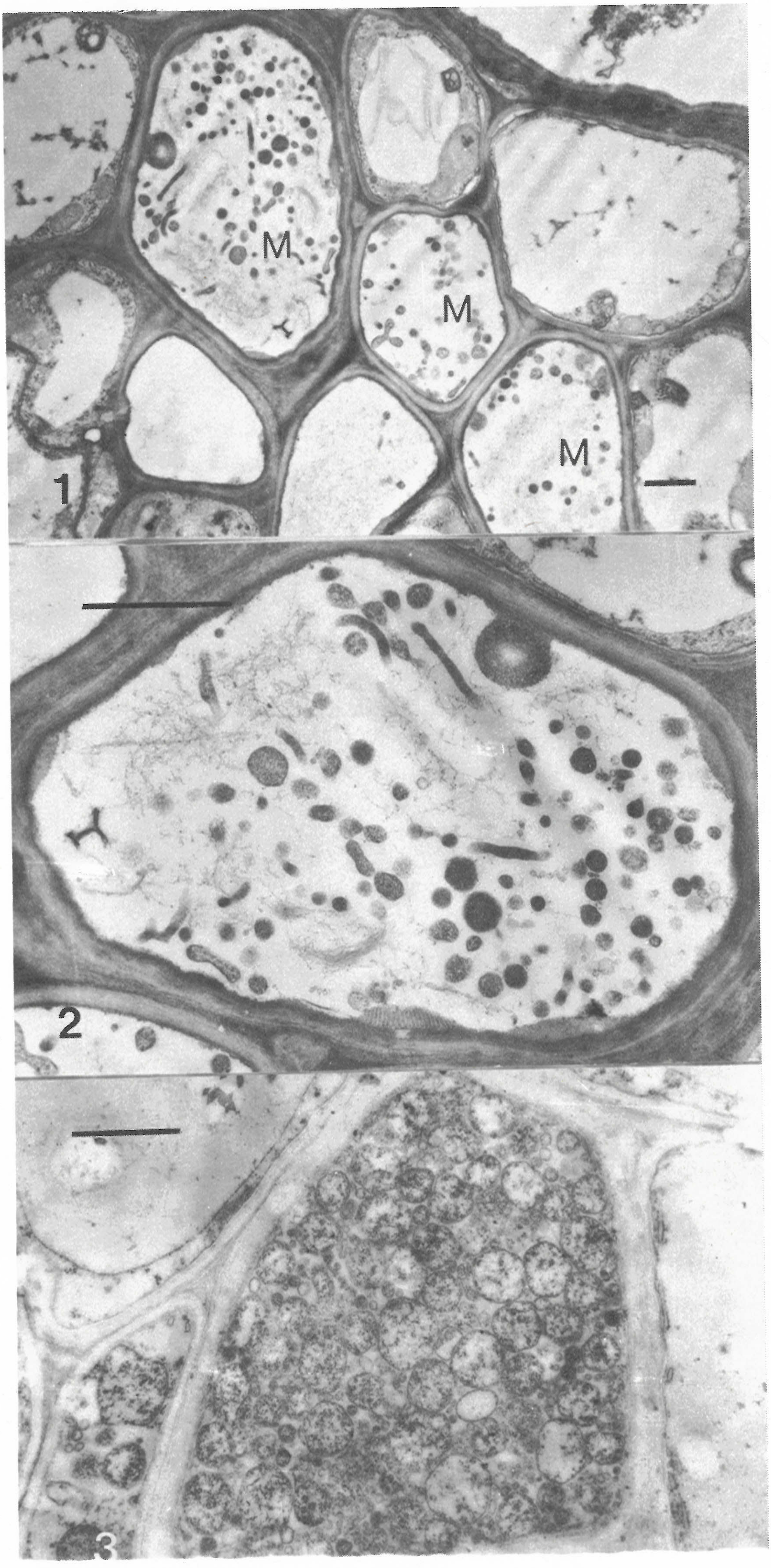
感染細花野牽牛簇葉病之長春草叢生病株及其對照長春草健株(圖 6)組織切片之染色與光學顯微鏡觀察結果，在病組織篩管細胞內看到極顯著之專一性藍色反應(Specific blue staining reaction)（圖4），在對照健全材料則無此項反應（圖 5）。各病組織篩管細胞內之藍色反應之形狀及濃度與分佈情形頗不一致，此項專一性藍色反應表示其 MLO 之存在及分佈情形，此項結果與電子顯微鏡觀察結果相符且與 Deeley *et al.* 與楊等之報告相符[6,8,12]，並證實 Dienes' stain 對 MLO 之專一性藍色反應[12]。

討　論

細花野牽牛簇葉病係第一作者在本省發現之新萎黃病[1,3,5,7,11]。本病在長春草上形成之極多數細葉與細分蘖叢生萎黃及早期枯死病徵顯然與甘藷、落花生、絲瓜等簇葉病迥異[4,8]，亦與泡桐及 Aster yellows 等已報告之簇葉病在長春草上之病徵不同[3,7,16,17]。本報告在病組織篩管細胞觀察到之多形性擬菌質體病原與土居等各項已報告之植物萎黃型病所發現之擬菌質體病原相近似[2,9,10,11,13,14,16,17]。本報告以本病之長春草病株組織之電子顯微鏡及光學顯微鏡觀察結果證實其病原菌質體之存在及其濃度與分佈位置並證實長春草為其病原菌體之良好且感受性高之寄主植物，本病易使長春草罹病枝枯且使全株枯死[7]。本病與本省田間發生之白花牽牛 (*Ipomoea sinensis* Choisy)、馬鞍藤 (*Ipomoea pes-caprae* Sweet)、山土豆 (*Alysicarpus nummularifolius* DC.) 等之簇葉病不同（未發表）。本病與其他作物之關係尚待探討。

誌　謝

本研究承行政院國科會資助 (NSC-71-0409-B055-02)，日本北海道大學四方英四郎教授，臺大蘇鴻基教授與農發會邱人璋博士之鼓勵與指導，本所林益昇主任之支持與吳淑雅小姐之協助，謹致謝忱。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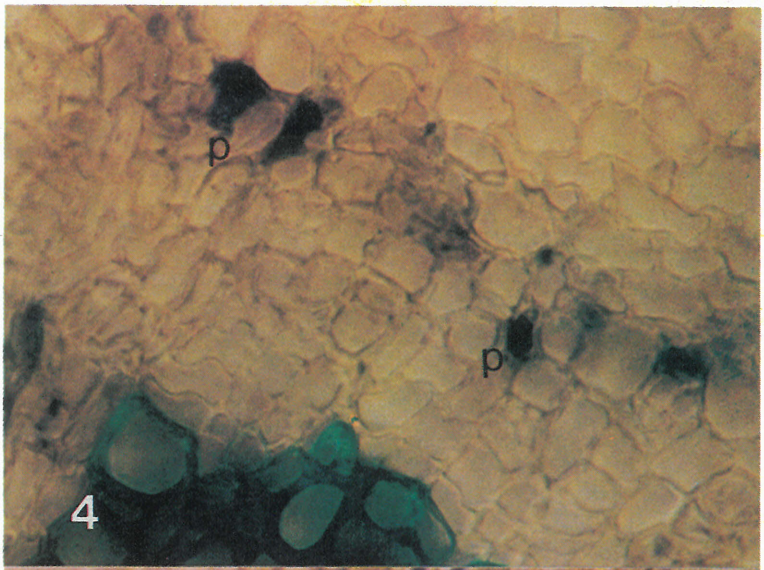
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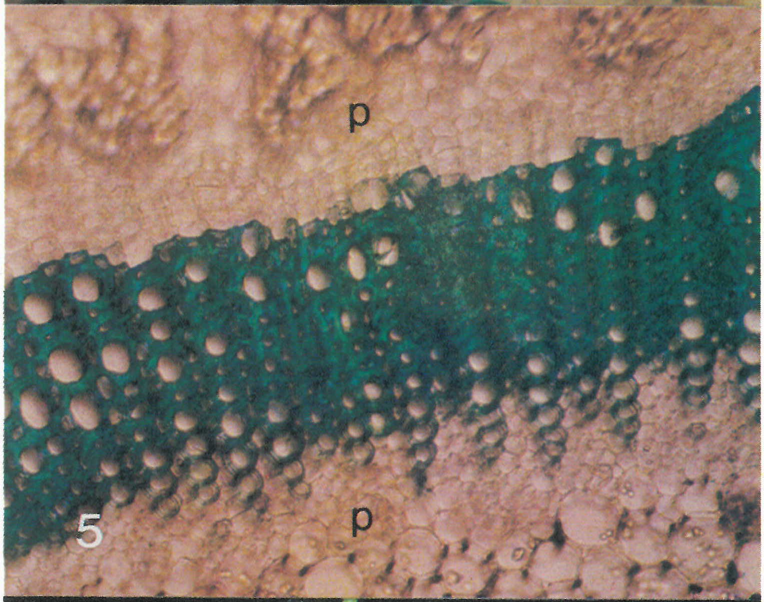
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圖片說明：

圖1.細花野牽牛簇葉病病葉韌皮部之超薄切片，在各篩管細胞內均可觀察到擬菌質體（M）。

Fig. 1. An ultrathin section of phloem of *Ipomoea obscura* leaf infected with witches' broom. Mycoplasma-like organisms (M) are seen in sieve cells.

圖2.細花野牽牛簇葉病病葉篩管細胞內（圖一之部份放大）存有橢圓形及曲管形等多形性菌體。

Fig. 2. Large magnificatoin of a part of Fig. 1. Illustrating spherical and filamentous forms of pleomorphic bodies in a sieve cell.

圖3.長春草病組織之篩管細胞內，聚集充滿許多大小不同之擬菌質體，菌體內可看到核糖體及網狀絲。

Fig. 3. Numerous mycoplasma-like organisms varied in size, are accumulated in a sieve tube cell of infected periwinkle leaf tissue. Ribosomes and net-like strands are seen in the bodies. (all the bars in the figures indicate 1μm)

圖4.長春草病株莖部組織切片之 Dienes' stain 染色，在其篩管細胞內（P）存有細花野牽牛簇葉病菌質體呈顯明之藍色反應。

Fig. 4. Transverse section of infected periwinkle stem treated with Dienes' stain, in whitch the phloem tissue (p) containing *Ipomoea obscura* witches' broom mycoplasmas stains distinctly blue.

圖5.長春草健株莖部組織切片之 Dienes' stain 染色，其篩管細胞內（P）並無藍色之染色反應。

Fig. 5. Transverse section of healthy periwinkle stem treated with Dienes' stain, none of the phloem (p) stains blue coloration.

圖6.感染細花野牽牛簇葉病之長春草病株（A）與其健株（B）。

Fig. 6. Periwinkle infected with *Ipomoea obscura* witches' broom (A), and healthy (B).

參考文獻

1. 四方英四郎・1972・海外に發生するマイコプラズマ様微生物による植物の病害・植物防疫，26：184～189。
2. 陳脉紀、曾德賜、楊一郎・1979・細花野牽牛簇葉病之電子顯微鏡觀察・植物保護學會會刊・68年年會論文摘要。
3. 楊一郎・1878・甘藷等三種植物簇葉病之兎絲媒介傳染研究・植物保護學會會刊・67年年會論文摘要。
4. 楊一郎・1979・甘藷簇葉病之兎絲媒介傳染・中華農業研究，28：23—28。
5. 楊一郎・1979・細花野牽牛簇葉病之研究・植物保護學會會刊・68年年會論文摘要。
6. 楊一郎・1982・臺灣數種植物萎黃病的光學顯微鏡觀察・科學發展月刊，10（1）：57—62。
7. 楊一郎・1982・細花野牽牛簇葉病之兎絲媒介傳染・中華農業研究，31（3）：235—238。
8. 楊一郎・1983・落花生簇葉病之兎絲媒介傳染與顯微鏡觀察・國科會科學發展月刊，11（9）：821—826。
9. 楊一郎、陳脉紀・1978・臺灣蕃茄簇葉病之研究（I）嫁接傳染與電子顯微鏡觀察・植物保護學會會刊，20（4）：360—365。
10. 楊一郎、蘇鴻基、呂淑卿・1973・臺灣芝麻簇葉病之研究（II）似菌質體病原・植物保護學會會刊，15（1）：1—4。
11. Chen, M. J. 1979. Electron-microscopic observations of several plant-infecting mycoplasma-like organisms in Taiwan. Plant Protect. Bull. (Taiwan), 21 : 25-36.
12. Deeley, J., W. A. Stevens, and R. T. V. Fox. 1979. Use of Dienes' stain to detect plant diseases induced by mycoplasma-like organisms. Phytopathology, 69 : 1169-1171.
13. Doi, Y., and H. Asuyama. 1981. Paulownia witches' broom disease. In "Mycoplasma diseases of trees and shrubs" pp. 135-145. (Tokyo University, Academic Press Inc.)

14. Doi, Y., M, Teranaka, K. Yora and H. Asuyama. 1967. Mycoplasma or PLT group-like micro-organisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Ann. Phytopathol. Soc. Japan 33 : 259-266.
15. Luft, J. H. 1961. Improvements in epoxy resin embedding methods, J. Biophys. Biochem. Cytol. 9 : 409-414.
16. Maramorosch, K., R. R. Gronados and H. Hirumi. 1970. Mycoplasma diseases of plants and insects. Adva. Virus Res. 16 : 135-139.
17. Miyahara, K., M. M. Tszaki, K. Tanaka and N. Sako. 1982. A New Disease of onion caused by Mycoplasma-like organism in Japan. Ann. Phytopath. Soc. Japan 48 : 551-554.

Light and Electron Microscopic observation of *Ipomoea obscura* witches' broom disease[1]

I. L. Yang[2], M. J. Chen and D. S. Tzen[3]

Summary

Electron microscopic examinations made on thin-sections of diseased leaves from *Ipomoea obscura* Ker. and periwinkle infected with *Ipomoea obscura* witches' broom revealed the presence of pleomorphic bodies of Mycoplasma-like organisms (MLO) in the sieve cells of phloem tissues.

These MLOs were found in various sizes and concentrations, and the size ranged from 60 to 740nm in diameter, and more aggregation or high concentrations in periwinkle diseased phloem tissues.

Light microscopic observation made on hand-sections of diseased leaves of periwinkle showed distinctly specific blue staining reaction with Dienes' stain in sieve cells, which indicated the presence of MLOs in the phloem tissues, and gave no reaction in phloem tissues and none of MLO was found from those sections of healthy plants. Since the morphology and localization and the specific reaction with Dienes' stain of these MLOs were similar to those found previously elsewhere in witches' broom diseases, these pleomorphic bodies seemed to be the causal organism of the new recorded *Ipomoea obscura* witches' broom disease.

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1. Contribution No. 1145 from Taiwan Agricultural Research Institute. This study was supported by Grant No. NSC-71-0409-B055-02, the National Science Council, ROC.
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中華農業研究（Jour. Agric. Res. China）33(1)：059~064（1984）

露期對稻熱病發病之影響[1]

簡　　錦　　忠[2]

摘要：稻熱病菌分生胞子着落於稻葉表面後，經發芽、產生附着器、侵入及病斑之形成，在此過程中，與氣象環境因子具密切關係。氣象因子中，除溫度外，露期（包括露水、雨水及點泌液等）之短久，直接影響病菌之侵入。在田間測定不同插秧密度間之罹病程度，顯示插秧密度愈密，即單位面積內稻叢數多（密）時，葉稻熱病愈早期發生，且其罹病程度亦愈嚴重。為進一步明瞭，各不同密度的稻叢內露期及露量之實際變化情形，利用 dew dynamics 測定水膜相對厚度（Relative wetness），在自然狀態測不出露水之環境下，行距與株距為 5 × 5 公分之小區葉面露期維持13.0小時，10×10公分區10.5小時，20×20公分區在上半夜具有水膜，此可能受微風之影響所以水膜厚度很低，40×40公分區就測不出露期。在露水箱內保持露期12小時比 8 小時者每支苗平均病斑數高出很多，而保持時間愈長，每支苗平均病斑數有愈增加的趨勢。由此可知稻熱病之發生，須要露水之存在，始能完成侵入之行動，同時露期最好保持12小時以上。

稻熱病之發病與蔓延，主要靠該病菌之分生胞子的傳播與感染。着落於稻葉表面之胞子，如在適當的條件下，發芽、產生附着器、侵入及病斑之形成，在此過程中，和氣象因子具密切關係，尤其是水滴（包括露水、雨水及點泌液等）。在田間露水常為水份之來源，露水在稻葉面上時間維持之長短，對病菌之侵入影響極大[8,10]。千葉等[2]及鈴木[7]指出，露水時間愈長，愈有利於侵入，連續10小時以上之露水，其侵入率要比 8 小時以內者高很多，又附着器形成率與雨量之相關關係很低。但降雨日數多時，因稻體的濕潤時間維持較長，有利病菌之侵入，故發病較多[3]。Barksdale and Jones[11]認為不同溫度下，稻熱病菌之侵入所需的露期時間差異很大，即愈適合侵入之溫度，其所需露期時間愈短。

本省稻熱病主要於第一期作的分蘗期至分蘗最盛期發生葉稻熱病，抽穗後發生穗稻熱病。據 Hashioka[12]報告，稻熱病之流行主要受溫度之影響，尚未提及露期之問題。但謝及梁[1]曾報告稻熱病菌分生胞子，若浸水後把胞子周圍水份吹乾，然後再供應水份，則已經發芽之發芽管常失去繼續伸長或產生附着器之能力。這種短時間浸水而影響胞子活力的現象，在田間幾乎每天均可能發生，晨間稻葉上的露水因太陽上升而乾燥，則就會發生此現象[6]。本研究係探討水膜相對厚度（Relative wetness）與稻熱病發病之關係，供作病害流行消長之依據。

材料與方法

一、插秧密度對水膜形成及發病之影響

供試品種為崑山五香梗（極感受性品種），插秧密度分為 5 × 5 、10×10、20×20、40×40公分

1.臺灣省農業試驗所　研究報告第1146號。本研究承國科會補助(NSC 71-0409-B055-03)之部分報告，特此誌謝。
2.本所植物病理系研究員。臺灣省　臺中縣　霧峰鄉。

等四個處理，每叢插秧 4 ～ 5 支苗。施肥量每10公畝基肥爲氮肥30公斤，磷肥30公斤，鉀肥10公斤。追肥量每10公畝僅用氮肥30公斤，分二次施用。其他管理按一般耕種方法。插秧後常巡視試驗田，記錄各處理區之病斑出現日期，病斑出現後每隔二天調查每叢的病斑（S. M 型）數一次。另利用 Dew dynamics 測定水膜相對厚度，比較各插秧密度間之差異。

二、露期保持時間與發病之關係

供試臺農67號，播種於直徑16公分，深19公分的塑膠盆內，待稻苗生長達本葉 3 ～ 4 葉時，舉行人工接種。接種用之稻熱病菌，係本系保存之菌株，並於 yeast starch agar 上培養繁殖二星期後，作成胞子懸浮液（5×10^{5}/ml），供接種之。另準備露水箱，在 165×255×245公分之大恆溫箱（外箱）內放置三個55×75×125公分之小恆溫箱（內箱），外箱溫度調節在 16°C，內箱箱底放入水盒，並調節溫度爲22°C，此狀態下內箱很快就形成露水，內箱即爲恆溫之露水箱。接種病菌後的秧苗搬入於露水箱內，保持葉片上的露水，經過一定時間（如表一內）後取出，放於溫室內一週後調查每支秧苗之病斑（S）數，同時在秧苗邊放置載玻片，其中央滴胞子液，測定胞子發芽率及附着器形成率。

結　　果

一、插秧密度對水膜形成及發病之影響

供試稻於 9 月 3 日插秧，在病斑出現一週前（9 月14～20日）之日平均溫度爲 27.4 °C，對病害發生雖略高，但晝夜最高、最低之差異達 7.0～9.4 °C 且日平均相對濕度89.0%，而一星期內每天相對濕度超過90%以上之時數爲13～15小時之多，頗有利病菌之侵染 [10]。故葉稻熱病於 5 × 5 公分區自 9 月21日出現病斑，爾後每隔 2 天調查每叢的病斑（M. S 型）數結果，病勢之進展頗迅速，即第一次（9 月21日）調查結果，每叢平均病斑數爲7.3個，第二次（9 月23日）11.5 個，第三次（9 月25日）18.8個，第四次（9 月27日）40.1個，最後（9 月29日）調查時，每叢平均病斑數已達51.2個之多，大部分葉片已開始枯死。10×10公分區自 9 月23日出現病斑，病勢進展曲線與 5 × 5 公分區略相同，但病斑數較少，即第一次（9 月21日）調查結果爲 0 個，第二次（9 月23日）5.5 個，第三次（9 月25日）12.4個，第四次（9 月27日）29.9個，最後一次每叢平均病斑數亦達35.2個之多。20×20公分區於 9 月29日才出現病斑，此時期在 5 × 5 公分區之被害葉已開始枯死，較10×10公分區之發病日期又遲延約一星期，10月 1 日之調查每叢平均病斑數僅爲16.2個而已。40×40公分區皆無病斑出現（圖一）。由此可知插秧密度愈密，即單位面積內稻叢數多（密）時，稻熱病愈早發病，且其程度亦嚴重，其原因頗複雜，但主要者，稻叢內的微氣象具有差異。尤其是露期之多少，及維持時間均不相同，如果露期維持較久，則有利於病菌之侵入。

爲進一步明瞭，各不同密度的稻叢內的露期及露量之實際變化情形，利用 dew dynamics 測定水膜相對厚度（Relative wetness）。即將該器的感應片，放置於稻叢之中間，另放置於試驗田畦畔上（大氣中，地平上約一公尺）作爲自然狀態下的水膜相對厚度。於10月 6 日及 7 日每天於17：00時開始啓用，晝間因測不到水膜，而停止測定。調節每30分鐘自動記錄一次，6 日之夜間在自然環境下無露水，但 5 × 5 公分及10×10公分區，自19：00時開始呈現水膜，其後水膜厚度直昇。5 × 5 公分區在20：00時已超過30%（已有利病菌之侵入），22：00時已超過69%，一直保持70～80%之間，最高達82%，翌晨08：00時下降至12%，則自出現水膜至消失之時間，計13小時之久。但自然狀態下有露水時（7 日測定）亦於20：00時超過30%，二夜測定結果，其直昇曲線極爲相符，而翌晨維持多 1 小時，而且達30%以上之時間，維持12小時以上，有利病菌之侵染。10×10公分區在20：30時超過30%，其上昇速度比 5 × 5 公分區緩慢，在00：30時才達到70%，其後維持至翌晨07：00時，其中最高爲

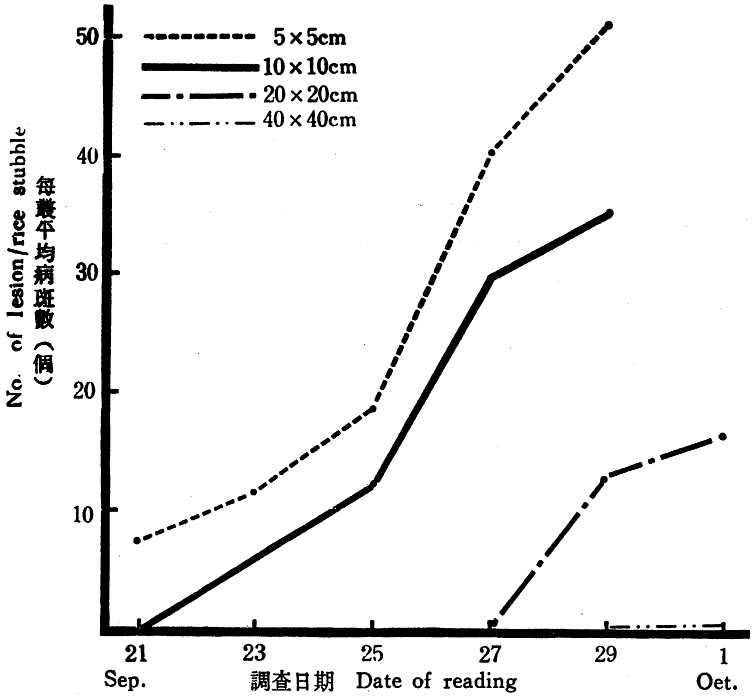

圖1. 插秧密度與稻熱病發生之關係

Fig. 1. The relationship between rice stubble density and blast disease severity.

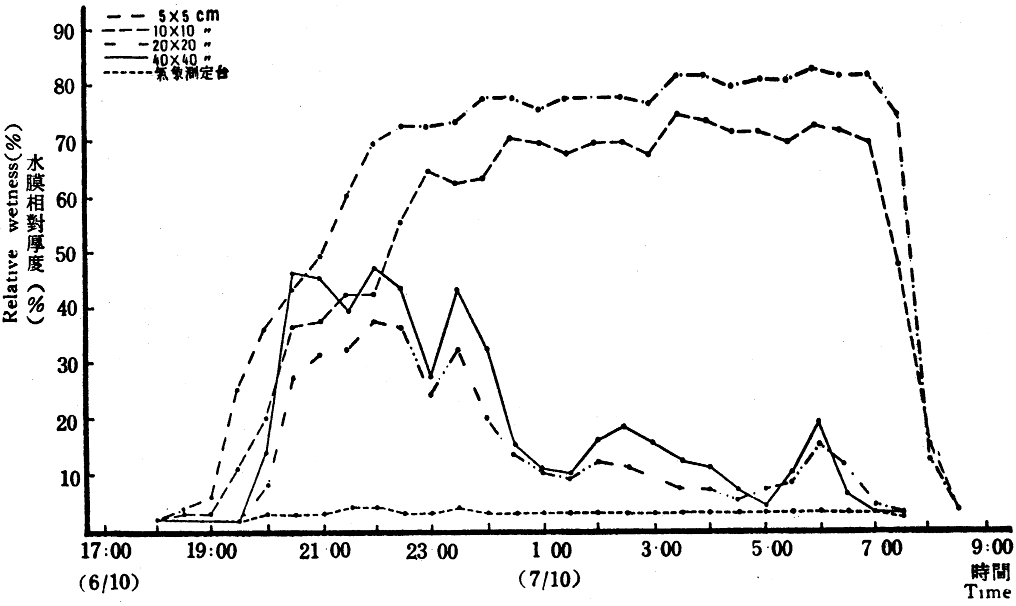

圖2. 插秧密度與水膜相對厚度之關係

Fig. 2. The relationship between rice stubble density and relative wetness.

74.0%，有露水時間與5×5公分區略相同。20×20公分區及40×40公分區自20：00時至24：00時均爲30—45%之間，其後漸次下降，或係由微風影響，翌晨08：00時已測不出水膜相對厚度。6日之對照區（大氣中，地平上約一公尺）自17：30—翌晨07：00時，一直保持5%以下，這表示測定當夜在自然環境下無露水（圖2）。

二、露期保持時間與發病之關係

稻熱病菌分生胞子着落於寄主組織表面後，在適當的環境下，逐漸伸出發芽管，而形成附着器，可完成侵入行爲。露水保持時間在4小時區，雖然胞子發芽率達54.6%，但附着器尚未形成，故葉片上皆無呈現病斑。自6小時區胞子發芽率提高達76.8%，但附着器之形成並不高，僅爲 5.5%，露水時間保持8小時以上時，胞子發芽率可達87.0%以上，10小時區已超過90.0%，而保持間愈久，胞子發芽率有徐徐昇高之趨勢，但其差異並不大。附着器形成率自露水保持時間6小時區開始，其維持時間愈長，形成率呈正比例漸次升高，24小時區之附着器形成率已達86.5%。因稻熱病發生由附着器產生侵入菌絲，直接侵入稻組織內細胞，而完成侵入[4,9]，故附着器之形成，爲決定病菌是否能引起感染寄主的條件。謝、梁[1]指出在 25°C 下，胞子遇水3小時後就開始形成附着器，但由本次試驗結果，在 22°C 下，露期維持6小時開始形成附着器，因謝、梁[1]係在葉片上病斑之稻熱病菌分生胞子作材料，在切葉片上作發芽試驗，結果在 16～32°C 的溫度下保持6小時，其發芽率可高達90%以上，而本次試驗所用之分生胞子，係於培養基上繁殖者，在 22°C 下，保持6小時的露期，其發芽率爲76.8%，稍低一些，此可能謝、梁是用來自寄主病斑上所得的分生胞子與培養基上所得胞子，兩者之間，發芽率已有差異之故。每株秧苗平均病斑數也隨露期保持時間愈長，愈增加病斑數。即10小時區每支苗平均病斑數僅有1.30個，12小時區4.57個，16小時區增加達9.75個，20小時區 10.29個，22小時區13.59個，24小時區高達 18.42個（表1）。

表1. 露期保持時間與發病之關係

Table 1. The relationship between dew period and infection

露期保持時間 dew period (hrs)	調查秧苗支數 No. of seedlings surveyed	總病斑數 total lesion	每支苗平均病斑數 No. of lesion/seedling	胞子發芽率 spore germiration (%)	附着器形成率 appressorial formation (%)
4	88	0	0.00	54.6	0.0
6	78	3	0.04	76.8	5.5
8	82	46	0.56	87.4	25.4
10	89	116	1.30	91.1	40.7
12	108	494	4.57	92.4	42.0
16	93	907	9.75	93.4	63.0
20	107	1,101	10.29	94.5	71.5
22	110	1,495	13.59	97.5	71.7
24	106	1,952	18.42	97.8	86.5

討 論

稻熱病之發生及蔓延消長過程中，和氣象環境因子具極密切關係。過去在氣象因子中認爲溫度比其他因子之影響大[5,12]，但近年來漸次明瞭露期之重要性[2,3,7]，在自然情況下，露水在稻葉面上維持的長短，影響病菌之侵入極大[8]。即露水時間長，有利於侵入，連續 12 小時之露水，胞子量之50%可完成侵入，其侵入率要比僅有8小時以內者高出很多[2,3,7]。由此可知，稻熱病菌胞子之發芽

、附着器之形成及侵入寄主之過程中，必須有露水存在，始能順利進行。在田間插秧密度，即單位面積內稻叢數不同時，雖了解稻叢間之微氣象有異，而稻熱病罹病程度亦不相同，但稻葉面上的露期長短與發病之關係，在本省尙無資料。據本研究得知，插秧密度愈密，稻熱病愈早期發病，且其程度亦愈嚴重。在自然界無露水情況下，密度5×5公分區，自19：00時開始出現水膜，至翌晨08：00時急下降，其維持露期時間達13小時之久，頗有利於病菌之侵入及蔓延。10×10公分區，呈現水膜遲 1.5小時，始達水膜厚度30%，其上昇速度亦較緩慢，而維持時間爲10.5小時，故發病程度較前者稍低。20×20公分區在上半夜略具有水膜之存在，但可能由微風影響，水膜相對厚度一直很低，同時發病亦較遲，較10×10公分區之發病日期遲延約一星期。40×40公分區可能稻株距過廣，通風良好，在自然界無露水時，亦測不出水膜厚度，且病害亦無發生。

另在室內露水箱內測定露期保持時間與發病之關係結果，保持時間12小時比8小時之每支秧苗平均病斑數高出很多，與加藤[3]及千葉[2] 等報告頗一致。而保持時間愈長每支苗之病斑數愈增加。至於胞子發芽率雖露期保持4小時有50%以上，但尙未形成附着器，無法完成侵入行動，保持10小時已有90%以上之胞子發芽率，附着器之形成亦提高很多，但病斑數比12小時少，雖然形成附着器，而大部份未能完成侵入之故。由本研究可證明稻熱病之發生，須露水之存在，始能完成侵入行動，同時露期最好須要保持12小時以上，可有利於病害之發生蔓延及猖獗。

參考文獻

1. 謝式坪鈺、梁文進・1978・稻熱病菌感染前之行爲，水稻病蟲害（生態與流行學）農復會刊行 199—212。
2. 千葉末作、千葉順逸、島田慶世、太田惠二・1973・人工降雨の時刻、强度および時間數といもち病（講要）日植病報 39(3)：187。
3. 加藤肇・1975・イネいもち病の疫學的硏究の現狀と問題點 植物防疫 29(6)：249—256。
4. 吉井甫・1936・稻熱病に關する硏究 Ⅱ病原菌の侵入法に就て 日植病報 6・・205—218。
5. 後藤和夫・1962・いもち病發生預察の技術的展望，病蟲害發生予察事業20周年記念誌 37—52。
6. 鈴木穗積・1969・いもち菌胞子の動態およびそれによる發生預察法 北陸農試報告 10：1—118。
7. 鈴木穗積・1974・いもち菌胞子の附着器形成と雨量（講要）日植病報 40(3)：188—189。
8. 橋本晃、酒井孝雄、平野喜代人・1975・試作結露計における水滴付着時間といもち病の發病（講要）日植病報 41(3)：242。
9. 橋岡良夫・1976・いもち病感染機構の電子顯微鏡的硏究 日植病報 42：237—238。
10. Asai, G. N., M. W. Jones and F. G. Rorie 1967 Influence of certain enviromental factors in the field on infection of rice by *Piricularia oryzae*. Phytopathology 57：237-241.
11. Barksdale, T. H. and M. W. Jones 1965 Minimum values of dew period and temperature required for infection by *Piricularia oryzae* (Abst.) Phytopathology 55：503.
12. Hashioka, H. 1950 Studies on the mechanism of prevalence on the rice blast disease in tropics, Tech. Bull. Taiwan Agr. Res. Inst. 8：1-255.

Studies on the Effect of Dew Period on the Severity of Rice Blast[1]

C. C. Chien[2]

Summary

The infection of *Pyricularia oryzae* on rice is closely related to dew period. Therefore, the dew dynamics was used to study the varying relationship between rice blast severity and dew period and/or dew quantity. The dew periods on the leaf surface were controlled by plant densities. Thirteen hours of dew period were observed in the plant density of 5×5cm, 10 hours in the density of 10×10 cm, and the relative wetness was very low in the density of 20×20 cm at late right. No dew was recorded in the density of 40×40 cm.

It comes to the conclusion that dew period which favored the infection of *P. oryzae* had better maintain more than 12 hours and dew period is positively correlated with the severity or lesion number of rice blast.

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1. Contribution No. 1146 from Taiwan Agricultural Research Institute. This study is supported by NSC research grant 71-0409-B055-03.

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Transmission of Citrus Likubin by a Psyllid, *Diaphorina Citri*[1]

C. H. Huang, M. Y. Tsai and C. L. Wang[2]

Abstract : In transmission studies of citrus likubin made during the years of 1981-1982, the psyllid, *Diaphorina citri* Kuway., successfully transmitted the likubin pathogen from diseased citrus plants to healthy Ponkan seedlings ; however, the efficiency of transmission was extremely low. Of 380 plants tested, only 5 plants showed likubin symptoms 1-2 months after test feeding. Leaf discs from the suspected plants, when grafted onto Ponkan indicator plants, induced likubin symptoms 2-4 months after inoculation indicating that the suspected symptoms are indeed due to likubin infection. Our preliminary results showed that new flush from diseased scions was a favorable inoculum source for acquisition feeding in transmission tests. The acquisition feeding time, infection feeding time required for successful transmission and the latent period in vector have yet remained to be determined.

(Key words : Citrus Likubin, Mycoplasma-like Organism, Vector Transmission).

Introduction

Citrus likubin, occurring in Taiwan since 1913[18], has remained to be the major threat to citrus industry in Taiwan. Most studies of the disease during the past decade dealt with electron microscopic demonstration of the causal agent[7,12,19,20] and the disease-curing effect of tetracyclines by tree transfusion[9,21].

Matsumoto and Su[15] reported the transmission of citrus likubin by using citrus aphid, *Toxoptera citricidus*, as vector. They concluded that the disease was possibly closely related to tristeza. This conclusion was generally accepted until the early 1970's when further investigations provided evidence that citrus likubin was not caused by a virus, but a Mycoplasma-like Organism (MLO)[7,20].

The citrus greening in South Africa and the stubborn in California are similar to citrus likubin in many respects[3,10,22]. However, the symptoms and electron microscopic studies showed that the likubin is more closely related to the greening[1,10]. Transmission of citrus greening was obtained in South Africa by using a psyllid, *Trioza erytreae*, as vector[16]. Similar diseases known as citrus die-back in India and leaf-mottling in

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the Philippines are transmitted by another species of psyllid, *Diaphorina citri* [14,17]. Chen et al.[8] found that there were numerous MLO bodies in mucous cells of salivary glands of adult psyllids of *D. citri* feeding for one month on likubin-diseased Ponkan plants. However, transmission of the disease by *D. citri* has yet to be substantiated.

This paper presents some experimental evidence of the transmission of citrus likubin from diseased Ponkan plants to healthy seedlings by *D. citri*. Parts of the results have been published elsewhere[13].

Materials and Methods

The transmission studies were conducted in 1981 and 1982. The citrus psyllids, *Diaphorina citri* Kuway., free of likubin pathogen, were reared on 2-yr-old Sunki seedlings in the greenhouse. The ability of the psyllid to transmit likubin was tested in the following experiment series :

(1) In the first experiment series, the source of likubin was collected from a Ponkan tree showing typical likubin symptoms in an orchard in Tung-shih, Taichung. Scions taken from the diseased tree were side-grafted onto 2-yr-old Ponkan plants on Sunki rootstock. Inoculated plants were kept in the greenhouse for one month before testing. A total of 10 inoculated plants in a cage of 18×18×30cm were used in this experiment.

Four groups of test insects, each comprising 2 to 3 hundred adults, were used in this experiment series. The first group was placed in the cage for one month starting about one month after the enclosed plants had been graft-inoculated with a likubin source. The test insects were then removed for transmission test. Upon the removal of the first group, the second group was introduced onto the same inoculated plants in the same cage. This process was repeated for the third and fourth groups.

After acquisition, the psyllids were allowed an infection feeding period of one month on test plants, 6 psyllids per plant. The psyllids were collected after the first infection feeding and transferred to new test plants for another month. After second infection feeding period, the psyllids that survived were collected and tested again.

(2) In the second series of experiments, the disease sources came from two locations : the Kuan-hsi Citrus Research Station in Hsin-chu and a Ponkan orchard in Tung-shih, Taichung. Scions were top-grafted onto Sunki rootstock. New flushes from diseased scions were used as inoculum sources for psyllids. Either nymphs or adults were used in the transmission tests depending on the maturity of the new flushes.

Four experiments were conducted in this series, i. e., PT-1, PT-2, PT-3 and PT-4, each using 8-10 diseased plants selected for same maturity of the new flushes to provide inoculum. The origins of inoculum sources for PT-1 and PT-2 were Ponkan and Liuchen trees from the Kuan-hsi Citrus Research Station, and for PT-3 and PT-4 were Ponkan trees from Tung-shih. The acquisition feeding and infection feeding were same as described in the first series of experiments.

(3) In the third experiment series, the psyllids naturally occurring on 2-yr-old diseased citrus plants outside the greenhouse were collected. About 15-20 nymphs or adults

were directly transferred to each test seedling and allowed to feed for 2 months.

All test plants were sprayed with insecticides after the psyllids had been removed. The test plants were then moved to greenhouse for observation of the symptom development. Plants showing likubin-like symptoms were indexed on Ponkan indicator plants by leaf-disc grafting (2). Those plants without symptoms were cut back after 8 months to enhance new growth and observation for symptoms continued for another 6 months.

Results

Transmission by adult psyllids feeding on likubin infected plants at various growth periods: In this experiment series, four groups of psyllids were used. Psyllids in the first group were tested after acquisition on 10 Ponkan seedlings during the first infection feeding period. Those that survived were collected and tested on 2 new seedlings for the second infection feeding. The psyllids that again survived were collectively tested on 1 seedling. Using the second group of psyllids, 25, 2 and 2 seedlings were tested in the three infection feeding periods, respectively. The corresponding numbers of test seedlings were 25, 4, 1 and 25, 0, 0 for the third and fourth groups of psyllids, respectively. Thus a total of 97 seedlings were tested in this experiment series. However, none was found with likubin symptoms. It seemed that adult psyllids could not acquire the likubin pathogen from the source plants.

Transmission by nymphs and adults feeding on new flushes: The results are shown in Table 1. Of the 260 seedlings tested, four plants, all fed on by test psyllids during the first transfer, showed likubin symptoms suspected to result from transmission by psyllids. The infected plants exhibited stunting and leaf yellowing 1-2 months after the psyllids had made test feeding on them. The most pronounced symptoms in these plants were leaf yellowing, sometimes with green islands on the yellowish leaves, zinc difficiency patterns on young leaves, and vein corking on mature leaves (Fig. 1, A and B). These symptoms were typical of citrus likubin observed in the field.

On indexing of those abnormal seedlings on 6-mo-old Ponkan indicator plants by leaf-disc graft method (2), likubin symptoms were observed in the indicator plants (Fig. 1 : C, D, and E) 2-4 months after inoculation. A test on Mexican lime seedlings gave no tristeza virus reactions. These results clearly indicate that the likubin pathogen was successfully transmitted by the psyllids from disease citrus plants to healthy Ponkan seedlings. However, the efficiency of transmission was extremely low.

Transmission by psyllids collected from 2-yr-old likubin infected citrus plants: Because of the low rate of transmission obtained in the above test series, a further experiment was performed. Table 2 showed the experimental results. Even though the number of the psyllids was increased to 15 for each test plant in the transmission tests, the rate of transmission did not improve. The only one plant of successful transmission was obtained when the test psyllids made acquisition feeding at their nymphal stage.

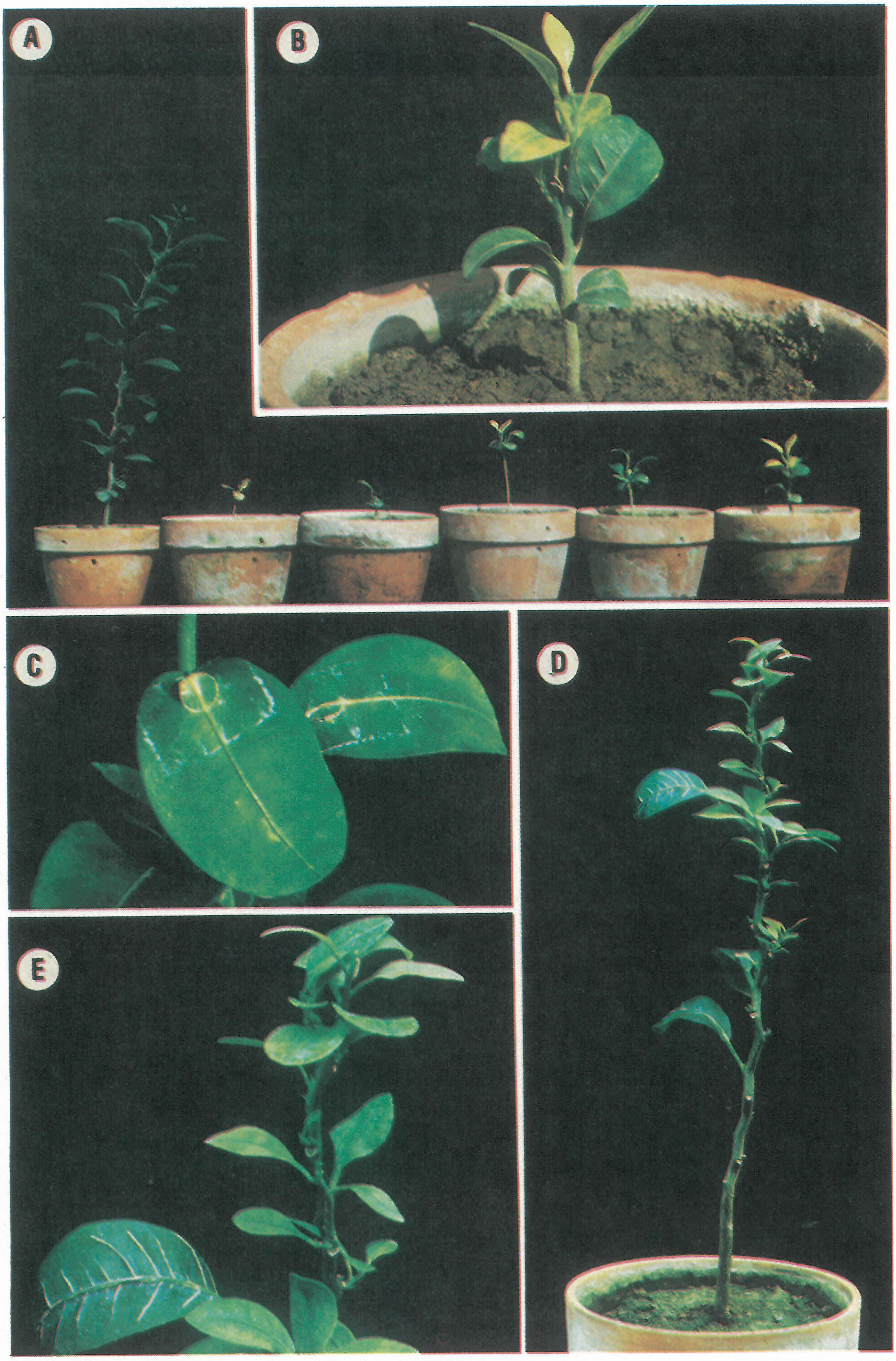

Figure 1. Symptoms of likubin on Ponkan seedlings. (A) Five plants to the right showed likubin symptoms 2–3 months after infection feeding by *D. citri*. The first plant from the left: healthy control. (B) Close-up of an infected seedling. (C) Ponkan indicator plant developed likubin symptoms 4 months after graft-inoculation with leaf tissues from psyllid-transmitted diseased Ponkan seedling. (D) Indicator plant showed typical likubin symptoms. (E) Close-up of the D plant. (Photographs of A, B and C were taken 4 months, and D and E taken 15 month after inoculation).

Table 1. Transmission of citrus likubin by *D. citri* that had previously fed for one month on new flushes from diseased scions

Exp.	Maturity of new flush	Developmental stage of psyllid	Infection feeding in serial transfers		
			1st transfer	2nd transfer	3rd transfer[a]
PT-1	1[b]	Nymph	1/12[c]	0/6	0/1
	5	Nymph	1/ 8	0/2	—[d]
	9	Adult	0/27	0/8	0/1
PT-2	3	Nymph & adult	1/15	0/4	0/1
	8	Adult	0/11	—	—
PT-3	1	Nymph	0/20	0/7	0/1
	5	Nymph	0/24	0/4	—
	9	Adult	0/25	—	—
PT-4	3	Nymph	1/17	0/7	0/2
	8	Adult	0/25	0/7	0/1
	12	Adult	0/24	—	—

a: The psyllids had an infection feeding period of one month on test seedlings after acquisition. They were then collected and transferred to new test seedlings for a second month of infection feeding. The survived psyllids were collected and tested again in the third transfer. During the infection feeding period, each test seedling was exposed to 6 psyllids.

b: Weeks after sprouting of new flush from diseased scions.

c: No. seedlings infected/total seedlings tested.

d: Not tested.

Table 2. Transmission of citrus likubin by *D. citri* collected from likubin-infected citrus plants[a]

Exp.	Developmental stage of psyllid	No. plants infected/total plants tested
1	Nymph	1/12
2	Adult	0/12

a: About 15 nymphs or adults were placed on each test seedling soon after they were collected from diseased plants outside the greenhouse. The test insects were allowed to remain on the test seedlings for two months.

Discussion

The spread of citrus likubin naturally in the field suggests that a vector must be involved. Su[(19)] stated that citrus likubin was probably transmitted by the oriental psyllid, but gave no details of the experiment. Tsai[(23)] and Yang, I. L. (Pers. Comm.)

obtained negative results in their studies on insect transmission of the disease. Therefore, the question about transmission of citrus likubin by a psyllid has remained unsettled. Recently, Chang [4] worked on 10 species of insects with sucking-mouth part to determine their possible role as likubin vector. These insects were directly collected from field and allowed a feeding on diseased plants for 5 to 30 days. Observing likubin symptoms on some of the test plants, he concluded that likubin was trasmitted by adult psyllids. However, Chang [4] did not index the suspected plants for verification. In the present study, we found that likubin could be transmitted by *D. citri* from diseased citrus plants to healthy Ponkan seedlings, although the efficiency of transmission was extremely low.

Capoor et al. [5] demonstrated that citrus greening in India was transmitted by *D. citri* with an acquisition feeding for 30 min or longer followed by an infection feeding for 1 hr or longer. Raychaudhuri et al. [17], and Catling and Atkinson [6] found that even a single psyllid was capable of acquiring the greening pathogen. Another psyllid, *T. erytreae*, vector of citrus greening in South Africa, became infective after 24 hr on diseased plant [5]. In contrast. the pathogen of likubin was very inefficiently transmitted by *D. citri* in Taiwan. Of the 380 seedlings exposed to the oriental psyllids that had made acquisition feeding, only 5 plants became infected. If likubin pathogen belongs to the greening-type, it may well be another strain of the greening pathogen which is more difficult to be transmitted by its vector than the greening pathogen found in India and South Africa. In addition, the uneven distribution of likubin pathogen in diseased plants [11] may possibly account in part for the low rate of transmission we observed.

Electron microscopic studies by Su and Leu [20], and by Huang et al. [12] showed that older leaves with vein corking symptoms had a higher concentration of likubin pathogen. In determining if the transmission is affected by the pathogen concentration in diseased plants, the plants used for acquisition feeding were divided according to growth periods after inoculation. However, our results did not show any positive correlation between pathogen build-up in inoculated plants and the ability of psyllid to acquire the likubin pathogen. In the first experiment series, adult psyllids had opportunities to feed on mature leaves of inoculated plants but they failed to acquire the pathogen.

It was reported that both nymph and adult of the psyllid were able to acquire the greening organism in India [5]. In South Africa, however, the nymph of *T. erytreae* seemed unable to transmit citrus greening [6]. Also, there was no evidence for transovarial transmission [5]. Because the acquisition feeding took longer time than it required for a nymph to become an adult in our experiments and because of the low rate of transmission obtained, the comparative efficiency of likubin by the psyllids at different developmental stages could not be determined although we observed all the five cases of successful transmission when the test insects were nymphs at the begining of the acquisition feeding.

The latent period of citrus greening in psyllids as determined in India was 8 to 12 days, and the psyllids retained infectivity throughout their life span [5]. In contrast, the vector became infective after one day on plants with greening symptoms in South Africa [5]. Our preliminary data suggest that *D. citir* is capable of transmitting the liku-

bin, and that the latent period was less than two months. Further work on likubin transmission is in progress.

Acknowledgements

The authors wish to express their thanks to Drs. R. J. Chiu, P. Y. Lai, Y. S. Lin, C. C. Yeh and B. H. Chen for help in the preparation of the manuscript. This study was supported by the Council for Agricultural Planning and Development under the projects 82-ARDP-4.1-A-13 and 82-ARDP-4.1-A-137.

Literature Cited

1. Bove, J. M., E. C. Calavan, S. P. Capoor, R. E. Cortez and R. E. Schwarz. 1974. . Influence of temperature on symptoms of California stubborn, South Africa greening, India citrus decline, and Philippines leaf mottling diseases. In L. G. Weathers and M. Cohen(ed.)Proc. 6th Conf. Intern. Organization Citrus Virol. p. 12-15, Univ. Calif. Riverside.
2. Blue, R. L., C. N. Roistacher, G. Cartia and E. C. Calavan. 1976. Leaf-disc grafting-A rapid indexing method for detection of some citrus viruses. In E. C. Calavan (ed.) Proc. 7th Conf. Intern. Organization Citrus Virol. p. 207-212, Univ. Calif. Riverside.
3. Calavan, E. C. 1968. A review of stubborn and greening diseases of citrus. In J. F. L. Childs (ed.) Proc. 4the Conf. Intern. Organization Citrus Virol. p. 105-117. Univ. Fla. Press, Gainesville.
4. Chang, A. F. 1980. Studies on the vector transmission of citrus likubin. Quarantine Monthly (Taiwan) 223 : 23-31. (in Chinese)
5. Capoor, S. P., D. G. Rao and S. M. Viswanath. 1974. Greening disease of citrus in the Deccan Trap country and its relationship with the vector, *Diaphorina citri* Kuwayama. In L. G. Weather and M. Cohen (ed.) Proc. 6th Conf. Intern. Organization Citrus Virol. p. 43-49. Univ. Calif. Riverside.
6. Catling, H. D. and P. R. Alkinson. 1974. Spread of greening by *Trioza erytreae* (Del Guercio) in Swaziland. In L. G. Weathers and M. Cohen (ed.) Proc. 6th Conf. Intern. Organization Citrus Virol. p. 33-39. Univ. Calif. Riverside.
7. Chen, M. H., T. Miyakawa and C. Matsui. 1971. Mycoplasma-like bodies associated with likubin-diseased Ponkan trees. Phytopathology 61 : 598.
8. Chen, M. H., T. Miyakawa and C. Matsui. 1973. Citrus likubin pathogens in salivary glands of *Diaphorina citri*. Phytopathology 63 : 194.
9. Chiu, R. J., M. Y. Tsai and C. H. Huang. 1978. Tree transfusion with tetracyclines as a measure for citrus likubin control. The ASPAC Food and Fertilizer Technology Center Book Series 13 : 91-103, Taipei , Taiwan.
10. Garnier, M., J. Latrille and J. M. Bove. 1976. *Spiroplasma citri* and the organism associated with likubin : comparison of their envelope systems. In E. C. Calavan (ed.) Proc. 7th Conf. Intern. Organization Citrus Virol. p. 13-17. Univ. Calif. Riverside.
11. Huang, C. H. 1979. Distribution of likubin pathogen in likubin-affected citrus plants. J. Agri. Res. China 28(1) : 29-33.
12. Huang, C. H, M. J. Chen and R. J. Chiu. 1980. Separation of a Mycoplasma-like Organism from the likubin complex in citrus. Plant Disease 64(6) : 564-566.

13. Huang, C. H., C. L. Wang and M. Y. Tsai. 1982. Transmission experiments of citrus likubin by insects. Plant Protection Bull. 24(4) : 282-283 (Abstract in Chinese).

14. Martinez, A. L. and J. M. Wallace. 1967. Citrus leaf-mottle-yellows disease in the Philippines and transmission of the causal virus by a psyllid, *Diaphorina citri*. Plant Disease Reporter 51 : 692-695.

15. Matsumoto, T. and H. J. Su. 1966. Likubin or Huanglungpin, citrus virus disease closely related to tristeza (1). J. Agri. Assoc. China, New series 56 : 1-12.

16. McClean, A. P. D. and R. E. Schwarz. 1970. Greening or Blotch mottle disease of citrus. Phytophylactica 2 : 177-194.

17. Raychaudhuri, S. P., T. K. Nariani, V. C. LeLe and C. R. Singh. 1972. Greening and citrus decline in India. In W. C. Price (ed.) Proc. 5th Conf. Intern. Organization Citrus Virol. p. 35-37. Univ. Fla. Press, Gainesville.

18. Sawada, K. 1913. Citrus likubin. The Formosan Agricultural Review 7(10) : 903-914.

19. Su, H. J. 1970. Further study on the pathogen complex causing likubin of citrus. Plant Protection Bull. Taiwan 12 : 190. (Abstract in Chinese).

20. Su, H. J. and S. C. Leu. 1972. Study on the pathogen complex causing likubin of citrus in Taiwan. I. Nature of Mycoplasma-like Organism associated with the disease. Proc. Natl. Sci. Coun. 5 : 109-126.

21. Su, H. J. and S. C. Chang, 1976. The responses of the likubin pathogen to antibiotics and heat therapy. In E. C. Calavan (ed.) Proc. 7th Conf. Intern. Organization Citrus Virol. p. 27-34. Univ. Calif. Riverside.

22. Schwarz, R. E. 1972. A review of stubborn and greening diseases of citrus. In W. C. Price (ed.) Proc. 5th Conf. Intern. Organization Citrus Virol. p. 1-5. Univ. Fla. Press, Gainesville.

23. Tsai, I. P. 1981. Study on vector of citrus likubin. (Unpublished).

木蝨媒介傳播柑桔立枯病之研究[1]

黄秋雄 蔡梅玉 王清玲[2]

摘要

室內飼養之四至五齡期木蝨若蟲或成蟲移至罹立枯病柑桔病穗之新生嫩葉上吸食一個月，再移出供傳播試驗。供試之三百八十餘棵柑桔苗中，有五棵呈現植株矮化，葉片黃化，老葉木栓化及新葉微量元素缺乏症。此種病徵與一般所稱之柑桔立枯病無異。病徵於木蝨吸食後約一至二個月呈現。自上述可疑柑苗切取葉片，以葉環組織接種法 (Leaf-disc graft method) 嫁接到椪柑指示植物，經二至四個月指示植物同樣產生立枯病病徵，證明立枯病病原由東方柑桔木蝨 (*Diaphorina citri* Kuway.) 蟲體傳到健全柑苗，唯木蝨傳播能力很低。其他有關研究，目前正進行中。

（關鍵字：柑桔立枯病、菌質病、媒介傳播）。

1.臺灣省農業試驗所 研究報告第1147號。

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Jour. Agric. Res. China (中華農業研究) 33(1) : 073~080 (1984)

Insecticide Resistance Study in *Plutella xylostella* (L.)

V. The Induction, Cross Resistance and Glutathione-S-transferase in Relation to Mevinphos-resistance[1]

by

Edward Y. Cheng, Tao-mei Chou and Ching-hua Kao[2]

Abstract : The diamondback moth, *Plutella xylostella* (L.), developed resistance to mevinphos rather slow and unstable. After twenty generations of mevinphos suppression at LC_{75} level, only 5-8 folds resistance was obtained. The mevinphos-resistant diamondback moth has a broad cross resistance to other organophosphorus compounds and cartap, but this resistance was not extended to most of the synthetic pyrethroids. Significant increase of glutathione-S-transferase activity occurred in the 4th instar and was paralleled with a reduction in mevinphos susceptibility during the same period. The age related glutathione-S-transferase activity change was also observed in the mevinphos-resistant strain except the overall enzyme activity was at higher level.

Introduction

Mevinphos is officially recommended for controlling the diamondback moth, *Plutella xylostella* (L.), in Taiwan. Several major vegetable cultivation areas have adapted this insecticide for many years due to its effectiveness and short residue period; undoubtfully, mevinphos has also confronted with resistance in the diamondback moth as any other synthetic insecticide does.

In a general survey performed in 1980-81 (4), we found that the mevinphos-resistance in the wild diamondback moth populations can still be considered mild in contrast with the high resistances to carbofuran and synthetic pyrethroids, despite they were introduced for field use much later. The resistance response of the diamondback moth to mevinphos is a very important matter but was never carefully studied before. Following the 1980-81 survey, we started to evaluate the induction rate and the cross resistance characters of mevinphos-resistance. The information will help us in understanding the consequences in regard to mevinphos application for the diamondback moth control

1. Contribution No. 1148 from Taiwan Agricultural Research Institute, the study is supported by NSC research grant 73-0409-B055-05

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and improving the undesirable aspects. Recently, we found the activity of glutathione(GSH)-S-transferase, a detoxication enzyme for organophosphorus, was increased in both the mevinphos- and profenofos-resistant strain diamondback moths [6] and the enzyme was investigated thoroughly in this study for its role and implication in relation to the organophosphorus resistance.

Materials and Methods

The IL-strain diamondback moth of our 1980-81 collections was used as the susceptible strain due to its high sensitivity to mevinphos. The IL-strain was also used as the parental strain for mevinphos resistance induction. Usually, one thousand late 3rd instar larvae were sprayed with mevinphos and the survived insects were collected for propagating the next generation. The press pressure was set at LC_{75} which was re-determined in each generation on 100 to 200 early emerged larvae of that generation. The increase of LC_{75} from generation to generation were counted as the resistance induction rate.

Fourteen commercially formulated insecticides listed in Table 1 were used for the cross resistance test. The cross resistance of mevinphos-resistant strain was studied in the same way as previously reported [7]. Insects were carefully selected and separated into four age groups, i.e., the 3rd, the early 4th, the late 4th instar larvae and the early pupal stage for GSH-S-transferase study. The enzymes were prepared and assayed as before [1,6] and the proteins were determined by the Lowry method [9].

Results

After twenty generations of mevinphos suppression at LC_{75} level, only 5-8 folds resistance was obtained in the diamondback moth. The resistance development was presented in Fig. 1 with suppression concentrations indicated in Y-axis and the diamondback moth generations in X-axis. The actual mortality following each press was noted in

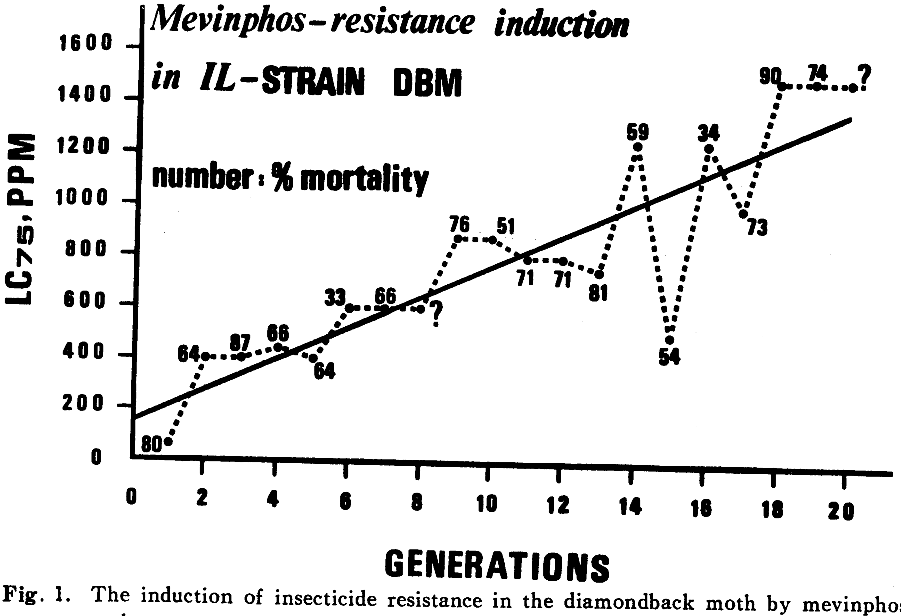

Fig. 1. The induction of insecticide resistance in the diamondback moth by mevinphos on each treatment at the selection pressure of LC_{75} with actual mortality indicated

the graph. Diamondback moth developed resistance to mevinphos much slower than that of carbofuran, cartap and fenvalerate(7). The mevinphos-resistant strain has obtained a level of resistance equivalent to that of the most resistant LC-strain(4) and is ready for the following tests.

The cross resistance of mevinphos-resistant strain to fourteen commonly used insecticides was listed in Table 1.

Table 1. The cross resistance of mevinphos-resistant diamondback moth to fourteen insecticides

Insecticides	S-strain		R-strain		R/S[a]	
	LC_{50}	LC_{75}	LC_{50}	LC_{75}	LC_{50}	LC_{75}
Organophosphorus						
Cyanofenphos	190	400	686	1,647	3.6	3.7
Diethquinalphion	230	500	1,289	2,800	5.6	5.8
Mephosfolan	360	900	608	1,521	1.7	1.7
Methamidophos	560	1,400	713	1,269	1.3	0.9
Phenthoate	670	1,700	589	1,793	0.9	1.1
Profenofos	100	230	444	1,040	4.4	4.5
Prophos	170	260	826	2,720	4.9	10.5
Prothiophos	310	560	643	1,475	2.1	2.6
Synthetic pyrethroids						
Cypermethrin	19.0	53.0	30.9	107.5	1.6	2.0
Decamethrin	3.6	11.0	12.4	44.2	3.5	4.0
Fenvalerate	8.3	23.0	15.2	31.2	1.8	1.4
Permethrin	14.0	30.0	38.4	65.4	2.7	2.2
UCX-33[b]	63.0	160.0	108.1	400.5	1.7	2.5
Others						
Cartap	290	580	1,804	4,203	6.2	7.3

a R/S for mevinphos-resistance is 5.6 for LC_{50} and 8.3 for LC_{75}
b Permethrin, formulated by Union Carbide Co.

In general, organophosphorus compounds were the most affected group by the cross resistance from mevinphos, and the effectiveness lowered significantly in four out of eight tested organophosphorus insecticides. Prophos was affected mostly and followed by diethquinalphion, profenofos and cyanofenphos; prothiophos, phenthoate, mephosfolan and methamidophos were not affected. Except decamethrin, the synthetic pyrethroids were not affected significantly. Unexpectedly, cartap was seriously affected by mevinphos cross resistance and its implication on cartap-resistance in field diamondback

moth populations needs to be evaluated further.

We have observed that the GSH-S-transferase activity is higher in the organophosphorus-resistant diamondback moth than in the susceptible IL-strain[6]. A more detailed study was carried out in both the mevinphos-resistant and the susceptible IL-strain diamondback moths and the results were presented in Fig. 2. The GSH-S-transferase may play a very important physiological role in the growth of the diamondback moth because the activity increased rapidly during the 4th instar and the activity remained high in early pupal stage. From the 3rd instar to the early pupal stage, the GSH-S-transferase activity increased ten times in the susceptible IL-strain,

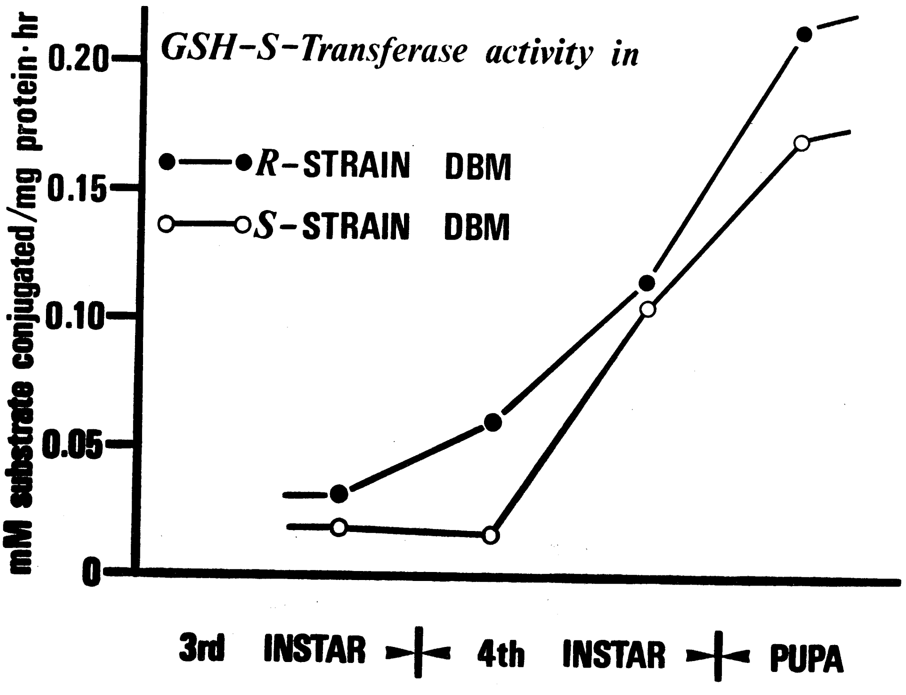

Fig. 2. Naturally occurred glutathione-S-transferase activity increases in the 4th instar larvae of both susceptible and mevinphos-resistant diamondback moths

and the same phenomenon was also observed in the mevinphos-resistant strain except at higher activity level (Fig. 2). We suspect this dramatic change of GSH-S-transferase may also altered the diamondback moth susceptibility to organophosphorus insecticides. In a subsequent test, organophosphorus susceptibilities at different larval instar were compared in the IL-strain diamondback moth, the results were presented in Table 2 along with the larval weight and body surface changes.

Table 2. The comparison of size, mevinphos susceptibility and GSH-S-transferase activity between the 3rd and the 4th instar diamondback moths

Instars	Weight (mg)	BodySurface (cm^2)	Weight/ BodySurface	LC_{50} (ppm)	LC_{50}/Weight/ Body Surface	Enz. Act.[a]
4th	5.8	0.160	36.3	632	17.4	0.106
3rd	1.6	0.072	22.2	108	4.9	0.019
4th/3rd	3.6	2.240	1.63	5.85	3.6	5.580

a. Enz. Act. : Glutathione-S-transferase activity in mM substrate conjugated/mg protein • hr

The larval weight increased 3.6 times from 3rd to 4th instar accompany with a body surface increase at 2.24 times. The morphological change increase the needed dosage by a factor of 1.63, but the actual increase is almost 6 folds. The discrepancy indicated that even the susceptible diamondback moth can become more resistant to organophosphorus insecticides by the age related physiological changes or as we have suspected, by the higher GSH-S-transferase detoxication rate.

Discussion

Chang has reported that field diamondback moth population from two intensively cultivated areas had high resistance to mevinphos and malathion[3]. Lee and Lee studied the organophosphorus resistance in the diamondback moth and found the diamondback moth collected from I-lan area was the most susceptible strain among ten areas they had sampled[8], which agreed with our 1980-81 survey[4]. They had found that the susceptibility was increased by rearing the diamondback moth indoors for several generations, but this phenomenon was not observed by us. They also induced a mevinphos-resistant strain having a resistant ratio of 10, which is similar to our result except that the press was performed on the adults rather than larvae. They observed high cross resistances in the malation-resistant strain to four other organophosphorus insecticides which agreed with our observation on mevinphos-resistant strain.

The slow and unstable development of mevinphos resistance in the diamondback moth may be a reason that made mevinphos still effective today in the diamondback moth control in many areas. The overall results of this study indicates that the diamondback moth can develop strong resistance to mevinphos and will result in serious cross resistance in organophosphorus compounds. The fact of both malathion and mevinphos can induce broad organophosphorus cross resistance may indicate that the diamondback moth have ability to generate a resistance which can respond to most organophosphorus insecticides.

After briefly comparing the structures of nine tested organophosphorus compounds, we found no sign of specific structure or functional group is responsible for the cross resistance. Instead, it seems that the whole organophosphorus group has lost its insecticidal action. The synthetic pyrethroids were not significantly influenced by the mevinphos cross resistance, only a three- to four-time increase in LC_{75} concentration was observed in decamethrin. We felt that the high synthetic pyrethroid resistance observed in fields was not related to the cross resistance from mevinphos.

Chang and Sun have concluded that the GSH-S-transferase was not involved in the detoxication of diazinon in the diamondback moth *in vitro* based on a soluble fraction

prepared more than an hour [2] ; we have found the same enzyme was deteriorated quickly after a long preparation period [6]. Wu and Sun also have found fivetime difference in GSH-S-transferase activity between the wild and susceptible strain, they decided not to correlate that to the observed organophosphorus resistance because no clear insecticide application history can be followed for the wild strain [10].

After noticed the increase in GSH-S-transferase activity in mevinphos-pressed diamondback moth, we felt that this enzyme should be studied carefully. We have found the diamondback moth having a dramatic GSH-S-transferase activity change in its late larval stage, which parallel with a decrease in organophosphorus susceptibility during the same period. This unexpected GSH-S-transferase activity increase is a very interesting matter in regard to our investigation of diamondback moth detoxication ability. We suspected the inherited physiological increase of GSH-S-transferase may have connections with the difficulty in inducing mevinphos resistance during the late larval stage. Another fact that we cannot overlook is that the diamondback moth can transform itself from a susceptible to a resistant individual simply by the age change. After dividing the 6 folds dosage increase by the morphological factor change of 1. 62, the susceptibility was reduced by a factor of 3.6 from 3rd to late 4th instar. During the same period, the GSH-S-transferase activity has increased 5 folds. We have no intention to interpret these two matters on their magnitudes change, but the possible correlation between them is worthy to be investigated.

Conclusion

Obviously, the slow and unstable induction of resistance in the diamondback moth by mevinphos has its advantage in pest management that we can justify the continuing use of this insecticide is still proper. Particularly, mevinphos degrades very fast on vegetable [5], which ensures the residue safety on marketed vegetables. Besides, the use of mevinphos will not interfere with the effectiveness of synthetic pyrethroids, which undoubtfully will be the major insecticides for vegetable usage in the future. However, we felt that the mevinphos resistance in the diamondback moth should be monitored closely in vegetable production areas because the cross resistance will jeopardize the effectiveness of other organophosphorus for diamondback moth control.

Naturally occurred GSH-S-transferase activity change in the diamondback moth larvae has made the usage of organophosphorus as control agent a very delicate matter. This study has revealed that the diamondback moth grown from the 3rd to late 4th instar was accompanied with a decline in organophosphorus susceptibility. Such a matter, to transform a susceptible population into a naturally immuned population, implied that the usage of organophosphorus insecticides in the diamondback moth control needs to be reevaluated. Particularly, the population composition of diamondback moth is usually a mixture in age groups. If the effectiveness of applied insecticide varied with the composition of age groups, the inconsistant effectiveness will affect the farmer psychologically and hence reduce their confidence on the officially recommended pest management methods.

References

1. Booth, J., E. Boyland and P. Sims. 1961. An enzyme from rat liver catalysing conjugations with glutathione. Biochem. J. 79 : 516-524.
2. Chang, C. P. 1977. A study on the mechanism of resistance to diazinon in the diamondback moth, *Plutella xylostella* (L.) Master thesis, National Chung-hsing University, Taichung, Taiwan, Rep. of China.
3. Chang, L. C. 1975. A study of resistance of *Plutella xylostella* (L.) to several organophosphorus insecticides. Plant Protection Bull. 17 : 431-432.
4. Cheng, E. Y. 1981. Insecticide resistance study in *Plutella xylostella* (L.) II. A general survey (1980-81). J. agric. Res. China 30 : 285-293.
5. Cheng, E. Y. and W. T. Lee. A systematical study of insecticide residue on vegetables. II. The persistence of eleven commonly used organophosphorus insecticides on cabbage leaves and glass surface. (unpublished information).
6. Cheng, E. Y., T. M. Chou and C. H. Kao. 1983. Insecticide resistance study *Plutella xylostella* (L.) IV. The activities of glutathione-S-transferase in the organophosphorus resistant strains. J. agric. Res. China 32 : 373-378.
7. Chou, T. M. and E. Y. Cheng. 1983. Insecticide resistance study in *Plutella xylostella* (L.) III. The insecticide susceptibilities and resistance response of a native susceptible strain. J. agric. Res. China 32 : 146-154.
8. Lee, S. L. and W. T. Lee. 1979. Studies on the resistance of diamondback moth, *Plutella xylostella* (L.) to commonly used insecticides. J. agric. Res. China 28 : 225-236.
9. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. biol. Chem. 193 : 265-275.
10. Wu, T. K. 1983. Relationship between resistance to organophosphorus and carbamate insecticides and acetylcholinesterase in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera : Yponomeutidae). Master thesis, National Chung-hsing University, Taichung Taiwan, Rep. of China.

小菜蛾抗藥性之研究

V、抗美文松品系誘發之交互抗性及其 Glutatione-S-transferase 活性變化情形

鄭 允 周桃美 高靜華

摘 要

採自宜蘭地區之感性品系小菜蛾對一般常用殺蟲劑感度均高，使用此品系測定對美文松抗葯性時，發現抗藥性之發展緩慢且不甚穩定，經過20世代，淘汰 75 %總蟲數之選擇，僅獲得5－8倍抗藥能力，較以往使用加保扶、芬化利及培丹誘發抗藥力爲緩慢。所得抗美文松品系小菜蛾對其他有機磷劑均能產生交互抗性，其中以普伏松之交互抗性最爲嚴重，其次爲拜裕松、佈飛松；美福松、達馬松及賽達松雖無顯著之交互抗性生成，然其原來之感度甚低，造成9種有機磷劑對抗美文松品系無良好殺蟲效果；對培丹亦有交互抗性，但對合成除蟲菊精類則無明顯之交互抗性發生。Glutathione-S-transferase 係一對有機磷類殺蟲劑之解毒酵素，其活性在抗藥品系中較高。研究中發現，該酵素在小菜蛾幼蟲期第四齡時有大幅度之變化，酵素活性由三到四齡增加5－6倍，四齡末以後至蛹期之酵素活性變化甚小，因此本試驗亦比較同爲感性品系之三、四齡幼蟲對美文松之敏感度，結果顯示該兩齡期幼蟲之敏感度隨 glutathione-S-transferase 活性的增加而降低，此一現象可能與解毒作用之自然增加有密切之關係，且對田間防治小菜蛾用藥劑量及其所能殺死之不同齡期小菜蛾幼蟲影響很大，因此在防治率之測定上，有值得再行檢討之必要。

1.臺灣省農業試驗所 研究報告第 1148 號。本計畫承國科會補助（編號 NSC73-0409-B055-05），謹致謝忱。

2.本所應用動物系研究員兼系主任、計畫助理及研究助理。臺灣省 臺中縣 霧峰鄉。

中華農業研究 (Jour. Agric. Res. China) 33(1) : 081～087 (1984)

百香果主要害蟲及害蟎發生調查[1]

溫宏治　李錫山[2]

摘要　百香果爲本省新興作物之一，近年來其栽培面積逐漸增加，民國 70—72 年經於本省主要產地所作調查結果，其主要害蟲有下列四種：(一)東方果實蠅〔*Dacus* (*Strumeta*) *dorsalis* Hendel〕於百香果園內全年發生，其棲羣高峰期在 7 —10月間，雌成蟲產卵於果實內，果實因受產卵刺傷而患部呈木質化，致皺縮成畸形，果實之大小與受產卵次數成顯著正相關係。(二) 紫偽葉蟎 (*Brevipalpus phoenicis* Geijskes) 全年發生，其棲羣密度在乾旱季節發生最多，雨量增加，其密度降低。成、幼、若蟎於葉片、枝蔓及果實表面上行銼吸取食，嚴重爲害時可導致葉片黃化、枝蔓枯萎，甚而落果。(三) 茶細蟎 (*Polyphagotarsonemus latus* Banks) 終年發生，以春季陰爽氣候較多，爲害新芽、新葉，致其變形、萎縮，影響發育。(四) 姬黃薊馬 (*Scirtothrips dorsalis* Hood) 於乾旱季節發生，爲害嫩心造成萎縮，影響生長。

百香果又名西番果或時計果，屬西番蓮科之蔓性熱帶果樹，原產南美之巴西，本省於日據時期自日本石川植物園引入紫色種，在本省各地試種，當時未受重視，後呈野生化而分佈於山地間 (3) 民國53年後自南美及中南美分別引進數種優良品種，頗受歡迎。鳳山熱帶園藝試驗分所於64年育成不需人工授粉品種後，百香果於本省始漸有大面積栽培。近年來之栽培面積在日漸增加中，由原先南投埔里地區至近來臺東地區均有專業化栽培，最近於臺南、高屏等地亦有小面積陸續栽培，逐漸成爲本省重要經濟果樹之一。

因百香果在本省尚屬新興作物，其有關蟲害方面之資料甚感缺乏，前人有記錄之害蟲種類僅有花薊馬、柑桔粉介殼蟲、球粉介殼蟲、黃介殼蟲及椿象一種等 5 種 (8) 而此等害蟲並非目前百香果之主要害蟲。百香果害蟲種類近年來經筆者調查之結果，已達 27 種之多，唯其中主要者爲東方果實蠅〔*Dacus* (*Strumeta*) *dorsalis* Hendel〕、紫偽葉蟎 (*Brevipalpus phoenicis* Geijskes) 、茶細蟎 (*Polyphagotarsonemas latus* Banks) 及姬黃薊馬 (*Scirtothrips dorsalis* Hood) 等四種 (5) 東方果實蠅及姬黃薊馬爲害其他作物前人已研究頗多 (1,2,4) ，但對百香果者尚少，紫偽葉蟎及茶細蟎在國內除曾、羅氏在分類上提出報告外，其他生態方面資料尚感欠缺 (6,7) ，故爲明瞭此等主要害蟲之發生情形，先作此田間棲羣調查，期能有助於未來防治工作之實施。

材料與方法

70年 7 月至72年 6 月進行以下各項試驗

一、東方果實蠅於百香果園活動及爲害果實調查：

東方果實蠅在百香果園活動調查係於鳳山本分所百香果園，設置 4 個誘殺器，每個誘殺器距離約

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1. 臺灣省農業試驗所　研究報告號1149號。本試驗蒙臺南商品檢驗局曾義雄課長鑑定害蟎種類，文成後蒙謝豐國博士斧正，謹此深致謝忱。

2. 本所鳳山熱帶園藝試驗分所助理研究員、副研究員兼系主任。臺灣省　高雄縣　鳳山市。

表1. 東方果實蠅產卵於百香果後化蛹調查

Table 1. The investigation on the pupation of the oriental fruit fly in the damaged passion fruits made by adult oviposition puncture

調查時期 Duration of investigation	調查之果實數 (No of fruits)						化蛹數 No. of pupa
	百香果大小(直徑、公厘) Size of passion-fruit in diameter (mm)					合計 Total.	
	10—20	21—30	31—40	41—50	51—60		
Ⅵ 1981	10	15	20	20	20	85	0
Ⅶ	10	20	10	15	15	70	0
Ⅷ	10	15	20	20	20	85	0
Ⅸ	10	16	22	20	20	88	0
Ⅹ	10	10	20	20	20	80	0
Ⅺ	10	20	20	20	20	90	0
Ⅶ 1982	15	15	15	15	15	75	0
Ⅷ	15	15	15	15	15	75	0
Ⅸ	15	15	15	15	15	75	0
Ⅹ	15	15	15	15	15	75	0
總計 Total	120	156	172	175	175	798	0

50m，內沾含毒甲基丁香油，置於園內4個角落，每週塡葯及收集果蠅一次。

東方果實蠅爲害百香果果實調查則每半個月至本分所百香果園中，作定期調查一次，每次抽樣果樹50株，每株逢機調查果實10個，計算被害果數及果實上受果實蠅產卵爲害孔數；每次調查並逢機採取大、中、小個體之各10—20個被產卵果實置於室內羽化器皿內作羽化觀察。並於70年10月間自果園採5—10，11—20，21—30，31—40，41—50，51—60 及 60 mm 以上直徑不等之果實各20個，調查被果實蠅產卵情形。

二、紫僞葉蟎、茶細蟎及姬黃薊馬之發生調查者：

每半個月至本分所百香果園內調查50株，每株調查嫩心、幼葉及老葉各10枚、莖部5枝，分別記錄害蟲種類及數量。

三、害蟲分佈調查：至本省百香果產地實地調查，以了解害蟲之分佈，俾使調查資料更充實。(註：氣象資料由本分所水土保持工作組提供)。

結果與討論

一、東方果實蠅(*Dacus dorsalis* Hendel)於百香果園活動及爲害果實調查

東方果實蠅在百香果園週年之活動，以夏、秋(7—10月)雨季最多，春季(3—5月)次之，冬季(1—2月)最少。由圖1所示其消長乃隨氣溫之高低及百香果之生育狀況而增減。本省南部東方果實蠅之寄主種類繁多，成蟲之出現終年可見，百香果園內東方果實蠅之棲羣密度受鄰近之寄主種類及栽培數量多寡而有影響，加以成蟲之活動力强，百香果園如鄰近栽植其寄主作物如檬果、番石榴、蓮霧………等，則爲害更烈。

百香果受東方果實蠅爲害與東方果實蠅之週年發生密度成正相關(圖1)，而百香果之果實盛產期均於果實蠅之高峰期，是故百香果容易受其產卵爲害。

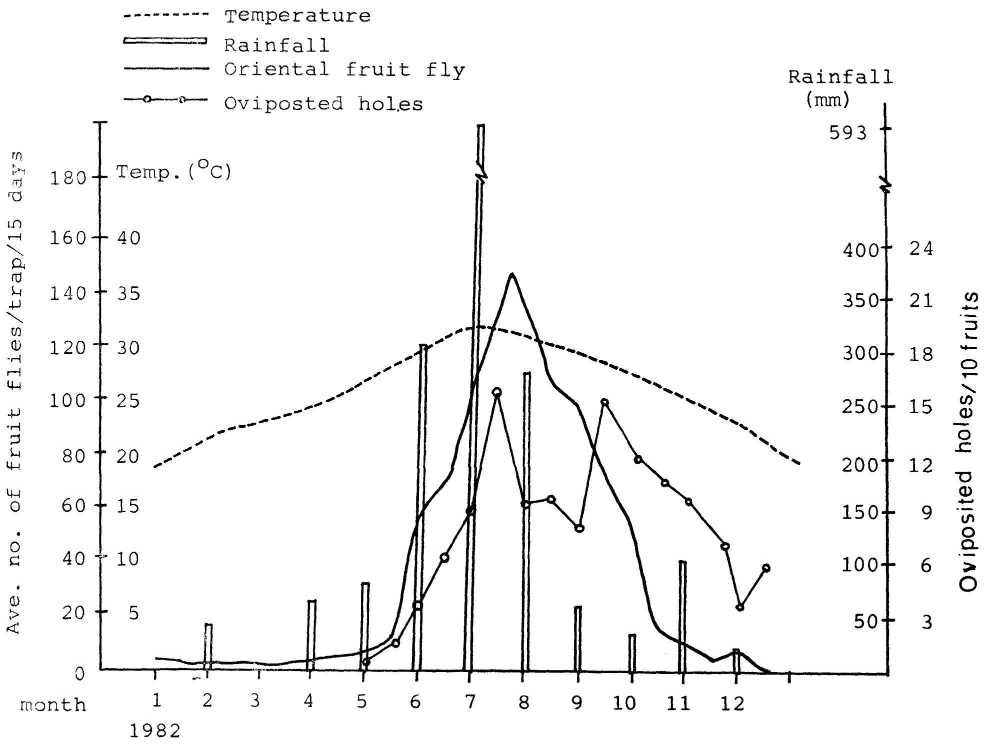

圖1. 東方果實蠅於百香果園之週年消長與產卵情形

Fig. 1. Seasonal occurrence of *Dacus dorsalis* and the oviposition on passion fruit

於70年6—11月及71年7—10月即百香果盛產期，前後共採取798個受果實蠅產卵爲害之果實，置於室內作篩蛹調查，但始終未見有東方果實蠅於沙中化蛹，採取部分果實內之卵，移接於成熟楊桃內飼養觀察至羽化，證實爲東方果實蠅之卵無誤，由此得悉東方果實蠅在百香果內很難存活，其原因或果皮堅硬幼蟲無法生活，或氰酸濃度高致使幼蟲死亡，此有待進一步調查。因此東方果實蠅主要在其他寄主果實上完成其幼期發育，成蟲飛入百香果園產卵於果實內，果實受產卵刺傷其傷口週圍漸成木質化，形如噴火口，致使果實皺縮、畸形，或促進果實早熟甚或引起落果。百香果受東方果實蠅產卵爲害之多寡，由幼果至成熟果，逐漸增加，其間關係如圖2所示成極顯著之正相關係。可能係愈近成熟之百香果香氣愈濃，增加引誘東方果實蠅來產卵，但其產卵爲害百香果，異於其他果樹，如檬果、柑桔均於成熟期受產卵爲害，而於百香果由幼果至成熟果均會被害，爲害幼果時，果實長大後形成畸形，影響產量與品質；爲害成熟果時，因幼蟲很難在果內生存，對產量影響較小，但對品質外觀的影響則大。

二、紫偽葉蟎（*Brevipalus phoenicis* Geijskes）田間發生消長

紫偽葉蟎之發生如圖2所示，於百香果園內全年均有發生，溫度之高低及雨量之多寡和葉蟎之發生消長均有顯著關係。在6、7月平均溫度超過27 °C以上，並且漸入梅雨期，葉蟎棲羣密度則可能受此高溫及多雨影響，逐漸減少，至9、10月後氣候逐漸轉爲乾旱，月平均溫度亦降至25°C以下，葉蟎棲羣密度亦漸增加，至12月間達高峰。成、幼若蟎主要分佈於百香果之葉、枝蔓或果實表面上行銼吸取食，嚴重時可佈滿被害部，致使葉片黃化、枝蔓枯萎，甚而落果。

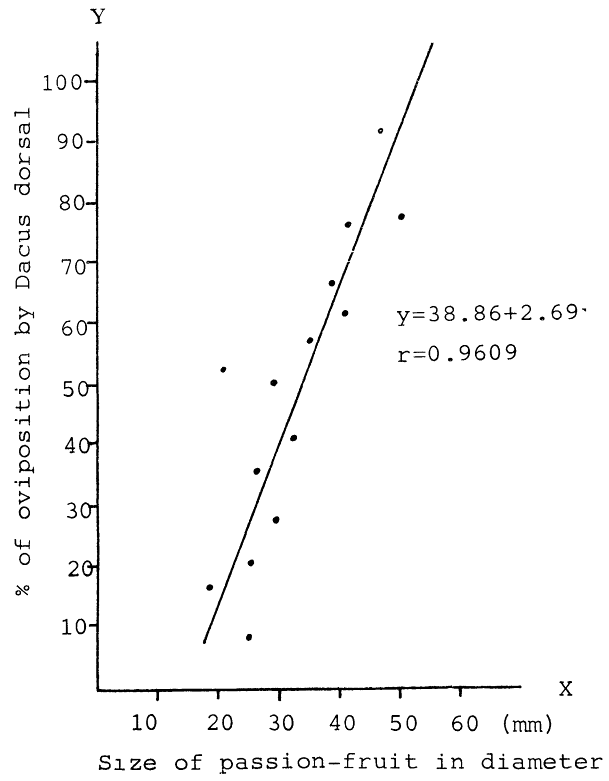

圖2. 百香果之大小與受東方果實蠅產卵之相關係

Fig. 2. The relation between the size of passion fruit and the percentage of oviposition by *Dacus dorsalis*

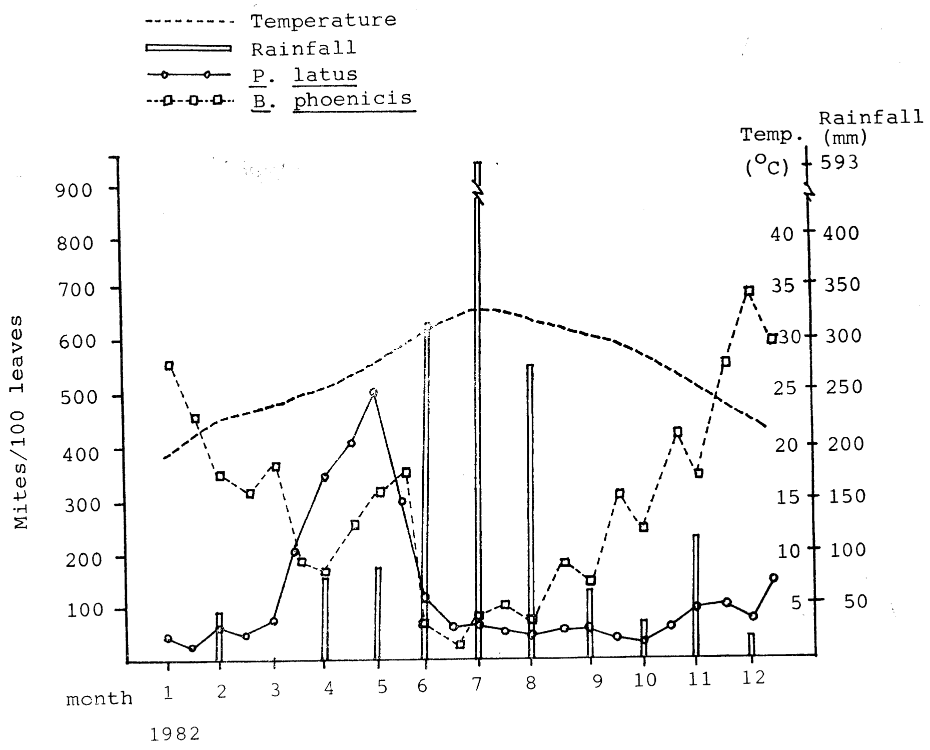

圖3. 百香果紫偽葉蟎及茶細蟎週年發生情形

Fig. 3. Seasonal occurrence of the mites on passion fruit orchard

三、茶細蟎（*Polyphagotarsonemus latus* Banks）田間發生消長

茶細蟎主要分佈於百香果之嫩梢或新葉上，其發生如圖3所示，週年不息，其棲羣密度受溫度高低及雨量之多寡所影響，於春季3～5月陰爽氣候較多。本蟎個體細小，肉眼難見，其爲害常導致新芽、葉變形、萎縮，生長停止，被害狀如罹毒素病，易被混淆。

四、姬黃薊馬（*Scirtothrips dorsalis* Hood）田間發生消長

姬黃薊馬主要分佈於百香果之嫩梢及新葉，銼吸患部有如熱水所燙傷，致使嫩梢枯萎，葉片黃化。其週年發生如圖4所示受雨量影響較大，溫度次之。1月間雨量最少，溫度亦低，其密度達最高峰，以後雨量及溫度漸高，其密度反逐漸減少，6～8月間雨量最多，溫度最高，則未發現其爲害，10月後雨量漸減，溫度漸降，氣候轉爲乾旱，其密度略增。

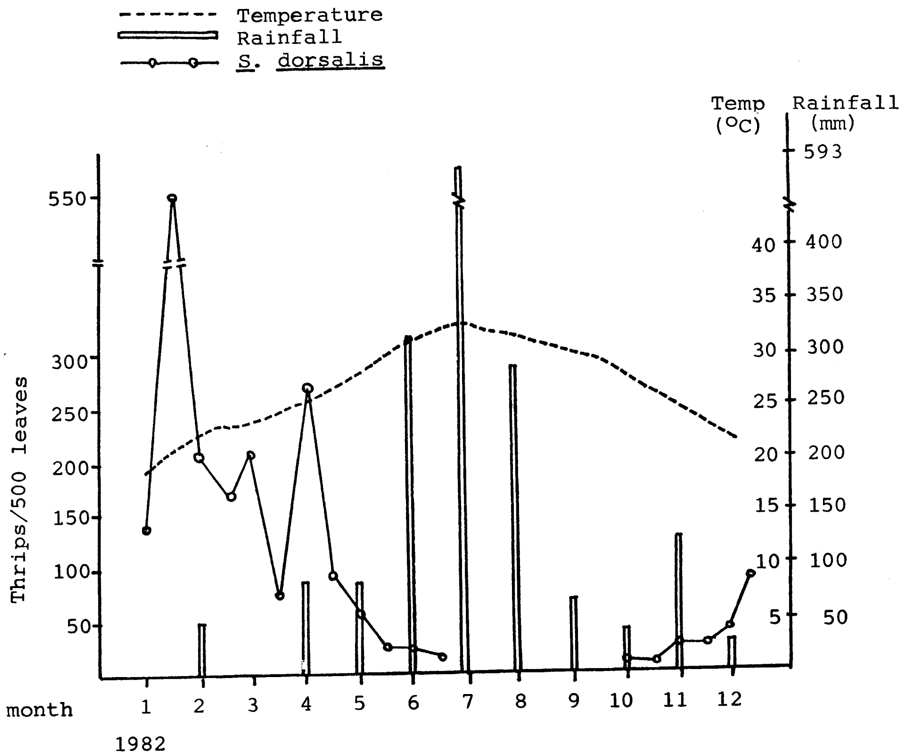

圖4. 姬黃薊馬於百香果園之週年消長情形

Fig. 4. Seasonal occurrence of *Scirtothrips dorsalis* on passion fruit orchard

五、百香果主要害蟲及害蟎於本省分佈情形

本省百香果最先於南投埔里地區有大面積栽培，後於嘉義、苗栗及高、屏等地有零星栽培，最近始於臺東地區有專業化栽培，有關百香果害蟲之分佈除受地域影響外，其種植時間長短、與鄰近作物種類均有密切關係。如表2，紫僞葉蟎於南投埔里地區較嚴重，而其他新植地區較少或無，臺東地區尚未發生嚴重，可能係埔里地區種植較久，該蟎累月適應積多成害。茶細蟎限於鳳山地區較爲嚴重，其他地區發生輕微，臺東尚未發現，此可能由於鳳山地區長久種植，由其他作物如木瓜等轉移感染所致。東方果蠅於本省普遍發生，百香果亦不例外，蓋其寄主植物繁多，有利於其棲羣發生。姬黃薊馬則於高、屏地區發生較多，可能來自檬果樹轉移爲害。

表2. 百香果主要害蟲及害蟎於本省之分佈情形

Table 2. The distribution of four major insect pests on passion fruit plant in Taiwan

地點 Location	紫偽葉蟎 *B. phoenicis*	茶細蟎 *P. latus*	東方果蠅 *D. dorsalis*	姬黃薊馬 *S. dorsalis*
苗栗三義 (Shani, Miao Li)	○	+	+	○
南投埔里 (Puli, Nan Tou)	+++	○	+	+
嘉義大埔 (Tapu, Chia I)	+	+	++	+
高雄鳳山 (Fengshan, Kao Hsioung)	++	++	+++	++
屏東里港 (Likang, Ping Tung)	+	+	+++	++
臺東卑南 (Painan, Tai Tung)	+	○	++	+

[a] 發生嚴重（70%以上受害）+++，中等（70～40% 受害）++，輕微（40～1%受害）+，無（○受害）○。+++ Sever-(infested over 70%), ++ medial-(infested between 70% and 40%), + light-(infested between 40% to 1%), Onone, Occurrence, respectively.

參考文獻

1.李文蓉。1982。利用雄蟲不孕性治蟲。行政院科技顧問組、植物保護研究聯繫協調小組報告P：292～297。

2.李錫山、溫宏治。1982。檬果薊馬類發生消長與爲害調查及其防治試驗。植物保護學會會刊。24（3）：179—187

3.林瑩達。1982。百香果栽培手册。鳳山熱帶園藝試驗分所P：1～2。

4.陳連勝。1981。臺灣網薊馬亞科之分類研究。植物保護學會會刊23（2）：117～130。

5.溫宏治、李錫山。1982。百香果害蟲種類及其棲羣發生調查。中華植物保護學會71年年會論文摘要。P：18。

6.曾義雄。1977。臺灣食葉性蟎之種類及其分佈情形研究Ⅱ擬葉蟎科。經濟部商品檢驗局臺南分局P：19～30。

7.羅幹成。1978。臺灣葉蟎類及防治方法對天敵之影響。中央研究院動物研究所專刊3：203～215。

8.蔡雲鵬。1965。臺灣害蟲名彙。臺灣省經濟部商品檢驗局，植物檢疫資料第5號P：22、30、75、82、93。

The Main Insect Pests on Passion Fruit in Taiwan[1]

Hung-chich Wen and Hsi-shan Lee [2]

Summary

Passion fruit is one of the new and flourishing crops in Taiwan. Its cultivated area has increased in recent years. Surveys on the pests were carried out from 1981-83. There are four major pests, *Dacus dorsalis* Hendel, *Brevipalpus phoenicis* (Geijskes), *Polyphagotarsonemus latus* (Banks) , and *Scirtothrips dorsalis* Hood. *Dacus dorsalis* occurred all year round in southern Taiwan and its population density peaked between July and October. The female adult punctured the fruits and laid eggs. Lignifications occurred around the wounded areas. Although the punctures usually stunted the fruits, no maggots were able to develop inside the fruits. There was a possitive correlation between the size of fruit and the time of egg lying, *Brevipalpus phoenicis* occurred year round. The mite was susceptible to precipitation, especially during the dry season. It appeared on the upper surface of a leaf, twig as well as the fruit and caused yellowing of leaves, dying back of the vine, and dropping of fruits. *Polyphagotarsonemus latus*, occurred all year round, especially during spring and cool weather and attacked the young terminal leaves causing them to be stunted, deformed and rugose. *Scirtothrips dorsalis*, occurred in dry season. This thrips attacked and stunted the terminal shoots.

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1. Contribution No. 1149 from the Taiwan Agriculture Research Institute.
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中華農業研究 (Jour. Agric. Res. China) 33(1)：088～093 (1984)

銷日菊花害蟲檢疫前處理—燻蒸法與燻煙法之利用[1]

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摘要：以燻蒸法 (Fumigation) 與燻煙法 (Smoking) 分別處理菊花害蟲—薊馬 (*Frankliniella intonsa, Thrips hawaiiensis*)、蚜蟲 (*Aphis gossypii, Myzus persicae*)、與二點葉蟎 (*Tetranychus urticae*)。燻蒸劑中以 32.3% 磷化鎂 (Magnesium phosphide) 11.5 g/m^3 燻蒸切花 12 小時或 15.4 g/m^3 燻蒸 8 小時，可使二點葉蟎全部死亡，以 2.1 g/m^3 燻蒸 8 小時或 6 g/m^3 燻蒸 1 小時，可使薊馬死亡。但影響之因子很多，效果不穩定。二甲苯 (Xylene) 可增强燻蒸之效果；以 8.5 g/m^3 之二甲苯加以磷化鎂 15.4 g/m^3 燻蒸 4 小時，薊馬、二點葉蟎全死，蚜蟲之死亡率提高至接近百分之百。

10種殺蟲劑與 7 種殺蟎劑之燻煙試驗中，以數種合成除蟲菊精藥劑對蚜蟲與薊馬有較優效果。殺蟎劑則未發現有對二點葉蟎效果理想者。以第滅寧 (Deltamethrin) 98% 工業原體 1 g/m^3 燻煙 1 小時，與磷化鎂 15.4 g/m^3 燻蒸 7 小時合併處理菊花，可同時使蚜蟲、薊馬與二點葉蟎全部死亡。

本省菊花害蟲種類很多，在植株各生育時期均會發生，如不予以適當防治，則會影響菊花正常之生育與菊花切花之品質，因此栽植菊花時田間害蟲防治一向為不可忽視之重要工作。外銷菊花因涉及進出口植物檢疫問題，對害蟲防治之要求較一般內銷者更為嚴格，除田間一般性防治外，往往在切花之後，尚需針對檢疫要求而進行一些特殊之害蟲處理。處理之對象主要是切花上之蚜蟲 *Aphis gossypii, Myzus persicae*、薊馬 *Frankliniella intonsa, Thrips hawaiiensis*、與二點葉蟎 *Tetranychus urtica*。此等害蟲在切花上經常發現，以一般田間防治甚難使之根絕。

以浸漬法處理切花為民間習用之方法，71年本所曾試驗16種殺蟲劑以及13種殺蟎劑用於浸漬切花之不同效果，當時曾篩選出 fenvalerate 與 dicofol 分別對薊馬與二點葉蟎有最佳效果 (1)。本試驗再度測試數種燻蒸劑處理切花害蟲之效果，並試以燻煙方式處理切花害蟲，企圖更進一步解決外銷菊花因害蟲存在而在檢疫上所造成之問題。本省菊花外銷之主要市場為日本，因此本試驗大部分係針對日本對進口植物檢疫標準而進行 (3)。

材料與方法

於71、72年自彰化縣田尾鄉菊花生產專業區採取生長狀況符合外銷日本標準之菊花切花樣品，品種包括黃秀鳳、英國紅、白鳥、韓大將、金日、雁之飛、岩鳳鏡、白粉、新大芬、大金等。採得之切花載運回本所實驗室，用不同之藥劑分別以燻蒸法與燻煙法處煙，觀察切花上薊馬、蚜蟲與二點葉蟎之死亡情形，以及切花是否受藥劑傷害。

燻蒸處理測試之藥劑為 E. D. B. (CH_2BrCH_2Br, azobenzol) ($C_{12}H_{10}N_2$), magnesium phosphide (Mg_3P_2) 與 xylene。其中 E. D. B. 與 xylene 均可於室溫 (22～25°C) 下自然氣化，

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azobenzene 需要加熱以使之氣化，magnesium phosphide 原本在室溫下即可緩慢釋放其中有效成分 pH_3，但在試驗中亦曾以加熱或將其投入水中使之較快速地釋放。

燻煙處理測試之殺蟲劑計有 deltamthrin 98% tech., fenvalerate 94% tech., flucythrinate 84% tech, permethrin 91% tech, mevinphos 100% tech, sumithion 95% tech, actellic 90% tech, baythion 85% tech, dursban 94% tech. phorate 10% G 等 10 種，殺蟎劑danicut 100% tech, dicofol 85% tech, binapacryl 95% tech, ethion 46.5% E. C., acrex 30% E. C., smite 55% E. C. azomite 60% E. C. 等 7 種。處理時將藥劑置於一塊加熱板上（200 °C以上），使原本為固態或液態之藥劑因高熱而轉變為懸浮於空氣中之細小之煙粒子。

各種藥劑均在室溫下於 26 l 之小型燻蒸箱中進行初步汰選，進一步之測試則於可設定溫度之燻蒸箱（240 l）或燻蒸室（7.7 m^3）內進行，燻蒸箱內均設有風扇或其他通風裝置，使其中藥劑得以充分擴散至每一角落。切花約佔容積 $\frac{1}{3}$ 左右。除了一些藥害嚴重或藥效不佳之處理，因結果明顯，故經過初悉之試驗即予淘汰外，一般之試驗均經過三次以上之重複。

結果與討論

一、燻蒸處理：

當溫度 17 °C 時，以加熱揮發之磷化鎂 32.3% 板劑 11.5 g/m^3 燻蒸切花 12 小時，可以消滅切花上之二點葉蟎，且於室溫下（25 °C）放置 24 小時，無蟎卵孵化（表 1）。將劑量增至 15.4 g/m^3，燻蒸時間縮短為 8 小時，可得到相同效果。繼續提高燻蒸劑之用量，則可能對切花有不利之影響。

表1. 以磷化鎂燻蒸菊花對二點葉蟎之效果 [a]

Table 1. Fumigation with magnesium phosphide against 2-spotted spider mites on chrysanthemum.

磷化鎂用量 Mag. phosphide 32.3% P. (g/m^3)	不同燻蒸時間之死亡率 Mortalities (%) at different exposureperiods (hr.)			
	4 小時	8 小時	12小時	16小時
3.8	—	—	—	92.2
11.5	78.2	80.0	100.0	+[b]
15.4	73.4	100.0	+	+
19.2	78.5	100.0	+	+
38.5	+	+	+	+

[a] 磷化鎂置於 200°C 以上加熱板約三分鐘，燻蒸箱 17°C. Magnesium phosphide was placed on hot plate over 200°C for about 3 minutes. Temperature in fumigation chamber was 17°C.

[b] 切花藥害 Phytotoxicity.

以磷化鎂燻蒸二點葉蟎，如處理之藥劑濃度不足或時間不夠時，處理完畢之當時，二點葉蟎可能僅呈現昏迷現象，經過數小時後，又再恢復活動。

以磷化鎂燻蒸使薊馬致死之劑量相當低，在 17 °C 下，以 2.1 g/m^3— 8 小時或 12.5 g/m^3— 1 小時之處理均可使切花上薊馬完全死亡（表2）。

表2. 以磷化鎂燻蒸菊花對薊馬之效果 [a,b]

Table 2. Fumigation with magnesium phosphide against thrips on cyhrsanthemum.

磷化鎂用量 Mag. phosphide 32.3% P. (g/m³)	燻蒸時間 Exposure period (hr)	死亡率 Mortality (%)
2.1	8	100.0
2.1	4	98.2
7.7	2	100.0
7.7	1	84.3
12.5	1	100.0
12.5	0.5	77.7

[a] 磷化鎂置於 200 °C 以上加熱板約三分鐘，燻蒸箱 17 °C Magnesium phosphide was placed on hot plate over 200°C for about 3 mintes. Temperature in fumigation chamber was 17°C.

[b] 處理之切花均無藥害 No phytotoxicity appeared on treated cutflowers.

磷化鎂對蚜蟲之燻蒸致死效果較差，以 21.0 g/m³ 燻蒸 8 小時，僅93.7%之蚜蟲死亡。此時切花有嚴重藥害（表3）。

表3. 以磷化鎂燻蒸菊花對蚜蟲之效果 [a]

Table 3. Fumigation with magnesium phosphide against aphids on chrysanthemum.

磷化鎂用量 Mag. phosphide 32.3% P. (g/m³)	燻蒸時間 Exposure period (hr)	死亡率 Mortality (%)	藥害 Phytotoxicity
12.6	8	62.5	−
16.8	8	81.8	−
21.0	8	93.7	+

[a] 磷化鎂置於 200 °C 以上加熱板約三分鐘，燻蒸箱 17 °C Magnesium phosphide was placed on hot plate over 200 °C for about 3 minutes. Temperature in fumigation chamber was 17°C.

磷化鎂對害蟲之致死能力與對切花之傷害受溫度影響極大。若溫度降低則害蟲（蟎）不易死亡，若溫度增高則切花易生藥害，因此燻蒸時之溫度必須充分控制，才能達到預期之結果。

二甲苯 (xylene) 對害蟲（蟎）燻蒸致死之能力並不特別强，如用量過高，亦會在菊花上造成傷害，處理過之葉片呈現黑褐色斑點。但因其有使蟲體昏迷的作用，因此可以配合其它燻蒸劑共同使用，而使燻蒸劑之用量或時間降低，當以 8.5 ml/m³ 之二甲苯配合磷化鎂使用時，僅需 16.6 g/m³ 之磷化鎂燻蒸 4 小時，即可使害蟲與害蟎全死（表4）。

表4. 二甲苯與磷化鎂共同使用之殺蟲效果

Table 4. Effects of fumigation with xylene and magnesium phosphide on chrysanthemum thrips, aphids, and 2-spotted spider mites.

二甲苯 Xylene (ml/m³)	磷化鎂 [b] Mag. phosphide 32.3%P. (g/m³)	燻蒸時間 Exposure period (hr)	死亡率 Mortality (%)			藥害 Phytotoxicity
			薊馬 Thrip	蚜蟲 Aphid	二點葉蟎Mite	
16.6	16.6	4	−	−	−	+
8.5	16.6	4	100.0	94.8	98.0	−
			(100.0)	(96.9)	(98.0)[b]	−
8.5	20.5	4	−	−	−	+

[a]. 磷化鎂置於 200 °C 以上加熱板約三分鐘，燻蒸箱 17 °C Magesium phosphide was placed on hot plate over 200°C for about 3 minutes. Temperature in the chamber was 17°C.

[b]. 括號中爲處理後第三日之死亡率 Mortalities in parenthesis were observed 3 days after treatment.

在不同使用濃度與處理時間下，Azobenzene（0.3 g/26 l—4 小時），EDB（0.1 ml/26 l—2 小時），會使菊花產生程度不等之藥害，而此時並未使蚜蟲、薊馬或二點葉蟎中任何一種害蟲完全死亡，因此這些藥劑均不適用於菊花害蟲之檢疫處理。

影響燻蒸處理的因子很多。某一因子之變異，即足以影響整個之結果。在許多條件之下，均不適合進行燻蒸處理，如有病斑、有蟲害傷口或其他機械傷害的菊花，燻後特別容易產生藥害。菊花品種間之差異亦大，一般白色與紅色花較黃菊易生藥害；而同色之不同品種間對藥害之反應並不相同。菊花之生理狀況不同亦影響燻蒸結果，莖粗者較莖細者耐藥。而氣溫高於 25°C 時，切割下之菊花亦易生藥害，氣溫愈低，植株可能因生理作用進行較緩慢，一般均較耐藥。此外，可能尚有許多其它尚未知曉之因子均在影響燻蒸之結果。

二、燻煙處理：

以燻煙法處理菊花害蟲，發現 10 種殺蟲劑中以合成除蟲菊精劑效果最好。以第滅寧（Deltamethrin）1 g/m³ 於密閉容器中燻煙處理切花 1 小時，可使切花上之蚜蟲與薊馬完全死亡（**表 5**）。如使用芬化利（Fenvalerate）40～80 g/m³ 處理 3 小時，亦有良好之效果。以 flucythrinate 處理過之切花易對人產生皮膚刺激之反應，而以百滅寧（Permethrin）與其它幾種殺蟲劑處理，則當昆蟲死亡時，切花已產生藥害。

表5. 以合成除蟲菊精劑燻煙處理菊花對蚜蟲與薊馬之效果

Table 5. Smoking with some synthetic pyrethroids against aphids and thrips on chrysanthemum.

藥劑 Chemical	用量 Dosage (g/m³)	燻煙時間 Exp. period (hr)	死亡率 Mortality (%) 薊馬 Thrip	死亡率 Mortality (%) 蚜蟲 Aphid	藥害 Phytotoxicity
第滅寧 Deltamethrin 98% tech.	0.4	2	97.6	98.8	−
	0.8	2	100.0	98.0	−
	1.0	1	100.0	100.0	−
	1.2	1	100.0	100.0	−
芬化利 Fenvalerate 94% tech.	20	3	80.4	85.4	−
	20	16	100.0	93.2	+
	40	3	94.3	93.0	−
	80	3	95.2	94.4	−
	120	3	97.2	100.0	+
百滅寧 Permethrin 91% tech.	8	2	89.5	72.0	−
	10	2	96.5	75.0	−
	16	2	100.0	86.0	+
	20	2	100.0	92.2	+

殺蟎劑中之大克蟎（Dicofol）過去曾以其 1000 倍稀釋液浸漬處理菊切花，對葉蟎類效果很好，但當以燻煙處理時，殺蟲效果未盡理想，以 40g/m³ 處理即會使葉片變的軟、黃、但以較輕之劑量處理，則葉蟎又未能死亡。經測試其它三種殺蟎劑亦均對菊花有不同程度之藥害，或使葉片轉呈黑褐，或使花部呈現污斑。

表6. 以第滅寧燻煙與磷化鎂燻蒸共同處理菊花之殺蟲效果

Table 6. Effects of smoking with deltamethrin and fumigation with magnessium phosphide on aphids, thrips and 2-spotted spier mites.

第滅寧 Deltamethrin 98% tech. (g/m³)	磷化鎂 Mag. phosphide 32.3% P. (g/m³)	處理時間 Exp. period (hr)	死亡率 Mortality (%)		
			蚜蟲 Aphid	薊馬 Thrip	二點葉蟎 Mite
1.0	—	1	100.0	100.0	—
—	15.4	5	—	—	100.0

[a] 磷化鎂置於 200 °C 以上加熱板約三分鐘，燻蒸箱 17 °C Magnesium phosphide wes placed on hot plate over 200°C for about 3 minutes. Temperature in the chamber was 17°C.

煙劑之毒性主要是由殺蟲劑粒子與昆蟲體接觸而產生。如爲蒸氣壓 (vapor pressure) 較高之藥劑，有時亦會釋出少量有毒氣體而產生燻蒸作用。因此，以煙劑處理昆蟲，可能同時具有觸殺與燻蒸兩種作用[4]。有些種類之殺蟲劑在加熱過程中會全部或部分被破壞、分解，因此並非任何種類之殺蟲劑均適於以煙劑使用。因此，同種藥劑以浸漬處理或以燻煙處理之殺蟲效果不盡相同。

煙劑之粒子一般多在半徑 1 μ 以下[5]，雖然極爲狹窄之花蕾、花萼等隙縫處亦可達到。藥劑以此種方式散佈完全不需加水，因而處理過之切花得以保持乾燥，免除了浸漬處理後，風乾之過程，並且亦減少切花之腐損，不致因處理而影響切花品質與瓶插壽命。一般燻煙處理並不限於在嚴格閉密之場所中才能施行，以一塊大型塑膠布將欲處理之物品覆蓋或將溫室之門窗緊密閉即可進行，當應用於檢疫處理時，爲確保每次處理後之效果相同，仍以於較密閉之容器中進行爲宜。

煙劑對蚜蟲與薊馬有效，但因無使二點葉蟎充分致死之能力，故與燻蒸劑混合使用方可達到害蟲與害蟎完全控制之目的，實驗結果證實合成除蟲菊精劑之燻煙與磷化鎂燻蒸合併施用具有雙重殺蟲效果，並可將處理之時間縮短，將達到標準之切花160～200枝 /m³ 置於 17 °C 之定溫燻蒸箱中，使用32.3%燻鎂死15.4 g/m³ 燻蒸切花 5 小時後，再於燻蒸容器中放入第滅寧 1 g/m³ 燻煙 1 小時，以這種綜合燻蒸與燻煙之方式處理過之切花上不再有任何活動之薊馬、蚜蟲或二點葉蟎，且於約 23 °C之室溫下，24小時之內無薊馬或二點葉蟎之卵孵化，對切花本身亦無任何明顯之不利影響。

結　論

菊花害蟲之檢疫前處理係針對日本對農產品之入境檢疫而施行，一切均以符合其檢查之標準爲主要目標。雖然日本爲順應其國內市場對菊花需要量之多寡，對害蟲檢疫有不同之水準；時而鬆馳時而嚴苛，但我方之處理仍應以能通過其嚴苛之檢查爲準，以避免可能之損失。

經過多次試驗，已經發現浸漬法中大克蟎1,000倍液浸漬切花對二點葉蟎有效[1]，燻蒸法中甲酸甲醇可消滅蚜蟲，乙烯乙酯可消滅薊馬[2]，而磷化鎂可同時消滅薊馬與二點葉蟎。燻煙法中則發現第滅寧之煙可使蚜蟲與薊馬死亡。然則無論浸漬法，燻蒸法或燻煙法均無法以某種藥劑單獨地將切花上之蚜蟲、或二點葉蟎全部殺死。合併已得之各種處理資料，以綜合之方式處理菊花，才能使其上之害蟲或害蟎充分死亡。

本試驗著重於利用燻蒸法與燻煙處理菊花害蟲，並試行單獨或合併數種藥劑進行處理，以謀求較少之藥害，較高之害蟲(蟎)死亡率。至於燻蒸、燻煙與浸漬法之混合應用，乃至田間切花前之各項保護措施之配合，目前則正繼續的進一步試驗探求中。

引用文獻：

1.王清玲，1982.菊花切花害蟲之防治，中華農研 31 (4) : 339—346。

2.王清玲，1983.菊花害蟲之檢疫前處理，花卉生產改進研討會專刊。出版中。

3.兒島司忠，1980.輸入檢疫之現狀，植物防疫 34 (18) : 339—346。
4 Busvine, J. R. 1971, Techniques for testing insecticides. p. 126-136. London.
5 Monro, H. A. U. 1961. Manual of fumigation for insect control. p. 216-220. Rome.

Study on the Quarantine Treatments of Insect Pests on Chrysanthemum Cutflowers— Fumigation and Smoking Methods[1]

C. L. Wang and R. T. Lin[2]

Summary

To fumigate chrysanthemum cutflowers with magnesium phosphide 32.3% P. at the concentration-time of 11.5g/m^3-12 hrs. or 15.4m/m^3-8 hrs. killed 2-spotted spider mites ; at 2.1 g/m^3-8 hrs. or 7.7g/m^3-2 hrs or 12.5g/m^3-lhr. killed thrips. To fumigate with xylene at the concentration of 16.6 g/m^3 and with magnesium phosphide 15.4g/m^3 for 4 hours killed thrips, aphids and 2-spotted spider mites at the rates of 100.0%, 96.9%, and 98.0%, respectively.

In smoking tests with 10 insecticides and 7 acaricides, some systhetic pyrethroids gave the most effective control on aphids and thrips. No acaricides were found effective for the quarantine treatment of spider mites. Treat culflowers with the smoke of deltamethrin 98% tech. at the concentration of 1 g/m^3 for 1hr. and with the gas of magnesium phosphide 32.3%.P. for another 5 hrs. killed thrips, aphids and 2-spotted spier mites completely.

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Vol. 33, No. 1 March 1984

JOURNAL OF AGRICULTURAL RESEARCH OF CHINA

(Formerly Journal of Taiwan Agricultural Research)

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Published by

THE TAIWAN AGRICULTURAL RESEARCH INSTITUTE

Taichung, Taiwan, Republic of China

行政院新聞局出版事業登記證局版臺誌字一四八七號
中華郵政臺字第二〇七六號登記為第一類新聞紙類